The Journal of Parasitology

Volume 39 JUNE, 1953 Number 3

STUDIES IN PANAMA *CULICOIDES* (DIPTERA, HELEIDAE) II. DESCRIPTIONS OF SIX ADDITIONAL NEW SPECIES[1]

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This paper is the second of a short series based on material collected by the junior author in Panama. Six new species were described in Part I (Jour. Wash. Acad. Sci., in press), and in succeeding papers now in preparation we intend to describe additional species, add notes on some of the little-known species of the Republic of Panama, and present a key for the determination of the species known to occur there.

Our terminology was explained briefly in Part I, and we should like only to repeat that we are using Tillyard's modification of the Comstock-Needham system for designating the wing veins, and that therefore the veins and corresponding cells called Cu_1 and Cu_2 by older workers are designated by us as M_{3+4} (cell M_4) and Cu_1, respectively.

Type specimens of new species are deposited in the U. S. National Museum, Washington, D. C.

Culicoides hertigi new species

(Figure 1, a–e)

Female: Length 1.0 mm., wing 0.8 mm. by 0.38 mm.

Head dark brown; eyes contiguous, bare. Antennae with flagellar segments in proportion of 15 : 12 : 12 : 12 : 12 : 12 : 12 : 12 : 22 : 22 : 25 : 25 : 35, distal sensory tufts on segments 3, 5, 7 and 11–15. Palpal segments (Fig. 1, c) in proportion of 6 : 15 : 15 : 5 : 8, third segment bulbously swollen, with large, deep, sensory pit, fourth segment much broader and shorter than fifth.

Mesonotum (Fig. 1, b) pruinose dark brown, with prominent pattern of large yellowish patches consisting of a large, submedian pair of elongate spots just before suture, a pair of narrow areas along humeral pits, and two pairs of small round spots in a transverse row on each side of submedian pair; large quadrate spots in prescutellar space pale and also a sublateral pair of rounded pale spots just ahead of and between wing bases. Scutellum brown in middle, yellowish on sides. Postscutellum dark brown; pleura pale, dark brown broadly across middle. Legs brown, narrow subapical bands on all femora, narrow subbasal bands on all tibiae and broad apical bands on hind tibiae, pale.

Wing (Fig. 1, a) with anterior radial cells both complete, short; costa to 0.6 of wing length; macrotrichia sparse along distal margin and in rows along veins on distal two-thirds of wing. Yellowish spots on anterior margin at wing base and over r-m crossvein small, the latter spot scarcely attaining anterior media; very dark areas between these two spots and over second anterior radial cell and most of first. Cell R_5 with an oblique double light spot at end of costa and slanting behind second anterior radial cell, an elongate spot, sometimes faint, along anterior margin of vein M_1 at this level, and a small, rounded spot in middle of cell R_5 at distal two-

Received for publication, August 29, 1952.

[1] Published under the auspices of the Surgeon General, U. S. Army, who does not necessarily assume responsibility for the professional opinions expressed by the authors.

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thirds. Cell M_1 with two elongated pale spots, the second not attaining wing margin; apices of veins M_1 and M_2 with short pale areas. Cell M_2 with small, oval, pale spots at mediocubital fork and just short of wing margin; cell M_4 with small, round light spot in middle near wing margin; anal cell pale at base and with two, separate, round, distal light spots. Halteres pale.

Abdomen dull brownish black; spermathecae two, subequal, very slightly pyriform.

Male genitalia (Fig 1 d, e): Ninth sternite with posterior margin not excavated, the posterior membrane bare; ninth tergite quadrate, short and broad, the apicolateral processes well-developed, triangular. Basistyles with ventral roots very slender and pointed, dorsal roots stout with rounded apices; dististyles slender and nearly straight, with abruptly bent, pointed apices. Aedeagus with basal arms slightly bent at mid-length, meeting two-thirds of total length of aedeagus, with a sharp, anterior cleft, distal portion stout with blunt, rounded apex. Parameres (Fig. 1, e) with bases stout and winglike, abruptly bent before the straight stems which taper by mid-length to slender filaments which are sinuately bent to fine pointed apices.

Holotype ♀, allotype, Rio Hato, Coclé Province, Panama, September 24, 1951, F. S. Blanton (light trap) (type No. 61504, U. S. N. M.). Paratypes: 1 ♂, 8 ♀♀, same data as type; 1 ♀, Las Tablas, Los Santos Prov., August 9, 1951; 1 ♀ Mojinga Swamp, C. Z., December 11, 1951.

The wing markings of this species are like those of the *debilipalpis* group except that there are two light spots in the distal part of the anal cell. This character will separate it easily from *C. dasyophrus* Macfie, which also has a mesonotal pattern of rounded patches, but *C. dasyophrus* also differs in having hairy eyes and one spermatheca. We dedicate this species with pleasure to Dr. Marshall Hertig, of the Gorgas Memorial Laboratory, Panama, Republic of Panama, a keen student of Neotropical biting Diptera.

Culicoides macrostigma new species

(Figure 2, a–d)

Female: Length 1.0 mm., wing 0.9 mm. by 0.47 mm.

Head dark brown; eyes contiguous, bare. Antennae with flagellar segments in proportion of 14 : 10 : 10 : 10 : 12 : 13 : 15 : 30 : 30 : 30 : 30 : 50, distal sensory tufts on segments 3 and 7–10. Palpal segments (Fig. 2, b) in proportion of 5 : 15 : 22 : 8 : 8, third segment swollen, with deep sensory pit with smaller pore.

Mesonotum dark chocolate brown, with prominent pattern of pruinose gray patches consisting of a pair of elongate, submedian spots, a pair of small humeral spots and two pairs of rounded lateral spots, one on each side of lateral suture. Scutellum brown in middle; pruinose gray on sides; postscutellum and pleura dark brown. Legs dark brown, forefemora and midfemora with subapical and all tibiae with subbasal, narrow, pale bands.

Wing (Fig. 2, a) with anterior radial cells complete, the second especially long, over twice as long as first; costa to 0.70 of wing length. Macrotrichia very scanty, confined to extreme apices of cells R_5 and M_1. Wing very dark brown infuscated, with discrete rounded light spots: base of wing pale, a large yellowish spot over r-m crossvein from anterior wing margin to base of anterior media; cell R_5 with spot at end of costa on anterior margin, a slightly triangular posterior spot slightly beyond and behind this one reaching nearly to vein M_1 and two small, rounded spots in line longitudinally behind second anterior radial cell, sometimes connected by a linear streak between their hind margins; cell M_1 with two rounded spots, the second far from wing margin cell M_2 with a double spot at level of mediocubital fork, two spots distad including an elongate subapical one and a large round one at apex of cell, a linear streak from wing base to subapical spot; cell M_4 with a round spot in middle of cell free from wing margin and anal cell with two separate, round spots in distal part of cell. Halteres pale.

Abdomen velvety brownish black; spermathecae two, subequal, pyriform.

Male genitalia (Fig. 2 c, d): Ninth sternite with very shallow mesal excavation, the posterior membrane spiculate; ninth tergite short and tapered, with very long, slender, fingerlike apicolateral processes. Basistyles with mesal margins minutely short-spinose, ventral roots very long, slender and straight with apices nearly contiguous and connected by a fine thread, dorsal roots stouter and about half as long; dististyles slightly curved, tapered to very slender, pointed apices. Aedeagus with broad basal arch about half again as broad as high, extending to about half of total length of aedeagus, distal portion stout and about twice as long as broad, almost parallel-sided, with truncated apex. Parameres (Fig. 2, c) with bases knobbed and bearing an elongate anterior process from knobs, stems very stout at bases and abruptly bent a short distance from bases, the distal portions nearly straight, directed, caudad and becoming slender, but extreme apices expanded and abruptly bent laterad in the shape of a beak.

Holotype ♀, Mojinga Swamp, Fort Sherman, Canal Zone, September 5, 1951, F. S. Blanton (light trap) (type No. 61505, U. S. N. M.) Allotype, same data except January 1952. Paratypes: 6 ♀♀, same data except dates, October 1951 to January 1952; 1 ♀, Loma Borracho, Canal Zone, October 29, 1951.

This species is very distinctive in the length of the second anterior radial cell and the two round light spots longitudinally in line behind it in cell R_5.

Culicoides miyamotoi new species

(Figure 3, a–e)

Female: Length 1.0 mm., wing 0.9 mm. by 0.45 mm.

Head dark brown; eyes bare, separated by width of a seta base. Antennae with flagellar segments in proportion of 15 : 15 : 18 : 18 : 18 : 18 : 18 : 18 : 18 : 18 : 18 : 27, distal sensory tufts single on segments 3 and 7, double on 8, triple on 9 and bordering an extremely broad, hyaline area on segment 10. Palpal segments (Fig. 3, c) in proportion of 5 : 20 : 25 : 10 : 15, third segment moderately swollen, with large deep sensory pit with somewhat constricted pore.

Mesonotum (Fig. 3, b) pruinose yellowish brown, with conspicuous pattern of punctiform dots at seta bases, a sublateral pair of dark brown spots just before suture. Scutellum yellowish brown in middle and on extreme ends on anterior side. Postscutellum dark brown, pleura dark brown in middle. Legs predominantly pale, knees dark brown and femora and tibiae with broad, median, brown bands; apices of fore tibiae brown.

Wing (Fig. 3, a) with anterior radial cells normal, short, costa to 0.6 of wing length; macrotrichia sparse on distal third of wing, none in anal cell. Wing brownish hyaline, small yellowish spots on anterior margin at wing base and over r-m crossvein, the latter spot just reaching over anterior media. Very dark areas between these two light spots and over second anterior radial cell and distal half of first. Cell R_5 with a small, round light spot at end of costa on margin, an elongate one bordering radial veins at level of vein R_{3+4}, an elongate one bordering base of M_1, a small round light spot in middle of cell between the first and second preceding, and a transverse spot not reaching wing margin just past spot at end of costa. Cell M_1 with two light spots far from wing margin, cell M_2 with a small spot behind medial fork, one just ahead of mediocubital fork, a round spot before wing margin and an elongate one halfway between the last two. Anal cell with light spot near base and a single round one near apex. Halteres yellow, anterior sides of knobs brownish.

Abdomen dull brownish black, distal margins of anterior segments pale on sides; spermathecae two, strongly sclerotized, subequal, pyriform.

Male genitalia (Fig. 3 d, e) : Ninth sternite narrow with shallow mesal excavation and low sublateral lobes, the posterior membrane bare; ninth tergite long and slightly tapered, the apicolateral processes rather long and slender and their bases broadly separated. Basistyles slender, ventral roots short and slender, slightly foot-shaped, dorsal roots about twice as long; dististyles long and slender, nearly straight with slender, rounded apices slightly bent. Aedeagus with low, broad, basal arch to a third of total length, basal arms stout, median portion gradually tapered to a blunt apex. Parameres (Fig. 10, d) with bases irregularly knobbed, stems nearly straight and slightly swollen midway, distal portions like curved rods with flattened, bluntly pointed apices directed across ventral meson.

Holotype ♀, allotype, Loma Borracho, Canal Zone, October 22, 1951, F. S. Blanton (light trap) (type No. 61506, U. S. N. M.). Paratypes: 1 ♂, 10 ♀♀, same data as types; 1 ♂, 2 ♀♀. Mojinga Swamp, Canal Zone, December 4, 1951.

In the very closely related species *C. ginesi* Ortiz from Venezuela and Panama the distal sensoria on the tenth antennal segment are composed of a row of three very small tufts, the third palpal segment is slightly broader, the spermathecae are more oval and not so pyriform, the wing lacks the basal pale spot in front of vein M_1 in cell R_5 and the subapical pale spot in cell M_2, and the male genitalia are quite different with strong distal spines on the broad, stout aedeagus. According to the male genitalia, these two species belong in the *copiosus* group. This species is named for Cpl. N. M. Miyamoto, an enthusiastic member of the junior author's survey team, U. S. Army Caribbean.

Culicoides gorgasi new species

(Figure 4, a–e)

Female: Length 1.2 mm., wing 1.0 mm. by 0.5 mm.

Head dark brown; eyes slightly separated, bare. Antennae with flagellar segments in proportion of 20 : 16 : 16 : 16 : 16 : 16 : 16 : 16 : 20 : 20 : 22 : 25 : 35, distal sensory tufts on segments 3 and 8–10. Palpal segments (Fig. 4, c) in proportion of 10 : 28 : 22 : 10 : 12, third segment swollen, with a small, deep, subapical sensory pit.

Mesonotum (Fig. 4, b) pruinose yellowish gray with pattern of dark brown punctiform dots at bases of mesonotal hairs; these brown dots more or less fused in a narrow median line and a pair of broad, sublateral bands from level of humeral pits almost to ends of scutellum; a few discrete dots in the elongate, submedian light discal patches and in the light, lateral areas before wing bases. Scutellum dark in middle. Postscutellum and pleura dark brown. Legs dark brown; knee spots blackish, all femora with subapical, all tibiae with subbasal and hind tibiae with apical, narrow pale rings.

Wing (Fig. 4, a) with anterior radial cells both complete, short; costa to 0.63 of wing length; macrotrichiae sparse on distal half of wing and in anal cell. Large yellowish spots at wing base and over r-m crossvein from costa to anterior media; broad, very dark areas between these spots and over second and distal half or first anterior radial cells. Cell R_5 with a small, round light spot at anterior margin at end of costa, another elongate one behind second anterior radial cell, and a large rounded, subapical light spot which frequently narrowly reaches wing margin. Cell M_1 with a long proximal, and an oval subapical, light spots, the latter occasionally attaining wing margin. Cell M_2 with pale streak from wing base to past level of pale spot in cell M_4, and another small round spot at wing margin. Light spot in cell M_4 broadly attaining wing margin and separated from veins of mediocubital fork. Ends of veins M_1 and M_2 with short pale spots. Pale area at wing base extending into base of anal cell, this cell also with two separate, distal light spots. Halter knobs dark.

Abdomen dark brown; spermathecae two, subequal, ovoid, the ducts not sclerotized.

Male genitalia (Fig. 4 d, e): Ninth sternite with broad mesal excavation, the membrane bare; ninth tergite long, the apicolateral processes long and slender. Basistyles slender, the ventral roots boat-hook shaped with apices nearly meeting mesad, the dorsal roots about as long and very slender; dististyles long and very slender, curved to pointed apices. Aedeagus cleft anteriorly to over three-fourths of total length, the anterior arms slender and nearly straight; apex slender and truncated, with a pair of very fine, pointed processes on posterior margins at level of fusion of basal arms. Parameres (Fig. 4, d) with bases knobbed, stems nearly straight, very slightly swollen, a very small ventral pouch just before the portion where the apices abruptly recurve and continue ventrocephalad as fine points each with two lateral barbs.

Holotype ♀, allotype, Las Tablas, Los Santos Prov., Panama, June 14, 1951, F. S. Blanton (light trap) (type No. 61507, U. S. N. M.). Paratypes: 10 ♀♀, same data as type; 7 ♀♀, Chitre, Coclé Prov., August 8, 1951.

The wing markings of *C. gorgasi* are of the type found in the difficult group of species related to *C. debilipalpis* Lutz, but the presence of two light spots at the apex of the anal cell will readily distinguish *C. gorgasi.* The punctate mesonotal markings are even more distinctive. This species is named in honor of General W. C. Gorgas, former Surgeon General of the U. S. Army and world-renowned leader of the crusades against malaria and yellow fever in the American tropics.

Culicoides mojingaensis new species

(Figure 5, a–f)

Female: Length 1.2 mm., wing 1.0 mm. by 0.5 mm.

Head dark brown; eyes contiguous, bare. Antennal proportions not measured, distal flagellar segments elongated. Palpal segments (Fig. 5, c) in proportion of 10 : 15 : 25 : 10 : 10, third segment swollen, with broad, shallow, subapical sensory pit.

Mesonotum (Fig. 5, b) rich dark brown; a pair of submedian, oval, yellowish brown spots; humeri each with a narrow, whitish pruinose line; sublateral margins on each side with two large, rounded, bluish gray, pruinose spots, the posterior one smaller and more or less continuous and concolorous with the flattened prescutellar area. Scutellum dark in middle. Postscutellum and pleura dark brown. Legs dark brown, all femora with subapical and tibiae with subbasal, and hind tibiae with apical, narrow, yellowish rings.

Wing (Fig. 5, a) with anterior radial cells normal, the second broad, costa extending to

0.65 of wing length; macrotrichiae very scanty on distal fourth of wing. Second anterior radial cell and distal third of first included in a very dark spot, which extends behind distal half of second as a small, round, dark spot in cell R_5, which in turn is margined by a U-shaped light spot extending from wing margin at tip of costa to posterior side of vein R_{4+5} cell R_5 also with a large, subapical, irregularly rounded pale spot broadly attaining margin; anterior half of wing with large pale spots at wing base, at level of r-m crossvein with a dark spot of subequal breadth between these extending halfway through cell M_2. Two pale spots in cell M_1; veins M_1, M_2 and M_{3+4} pale margined on about distal halves. Cell M_2 with base pale, a geminate light spot at level of mediocubital form and two pale spots toward wing margin; cell M_4 with a large, round light spot; anal cell pale at base and with two separate distal light spots. Halter knobs dark.

Abdomen dull dark brown; spermathecae (Fig. 5, d) two, subequal, slightly ovoid, bases of ducts sclerotized but not tapered to spermathecae.

Male genitalia (Fig. 5e, f): Ninth sternite short with very shallow mesal excavation, the posterior membrane spiculate; ninth tergite long and tapering, the apicolateral processes long and slender, about half as long as the distance between their bases. Basistyles long and slender, their mesal sides with short, stout spines; ventral roots stout and boat-hook shaped, dorsal roots slender and simple; dististyles slender, nearly straight until their abruptly incurved, pointed apices. Aedeagus with basal arch a little more than half of total length, the basal arms slender and curved; distal portion with rounded, striated apex and a pair of narrow, pointed flanges on sides from shoulders to about halfway to tip. Parameres (Fig. 12, e) with bases knobbed, stems slender and slightly sinuate, apices curved ventromesad and ending in sharp, needlelike points each with four lateral barbs.

Holotype ♀, Monjinga Swamp, Fort Sherman, Canal Zone, August 28, 1951, F. S. Blanton (light trap) type No. 61508, U. S. N. M.). Allotype, same data except September 4, 1951. Paratypes: 14 ♂♂, 33 ♀♀, same data except dates August 28, 1951, to February 1952; 1 ♂, 3 ♀♀, Loma Borracho, Canal Zone, October 23, 1951.

This species resembles *C. tricoloratus,* n. sp., in having both yellow and bluish pruinose markings on the mesonotum, the wing veins M_1 and M_2 extensively pale margined, and in the structure of the male genitalia. However, *C. tricoloratus* is much smaller, without the small, round, dark spot cut off behind the second anterior radial cell, with the third palpal segment more swollen, the membrane on the ninth sternite not spiculate, the apicolateral processes of the ninth tergite short and the stems of the parameres more swollen.

Culicoides tricoloratus new species

(Figure 6, a–e)

Female: Length 1.0 mm., wing 0.95 mm. by 0.45 mm.

Head dark brown; eyes well separated, bare. Antennae with flagellar segments in proportion of 18 : 15 : 15 : 18 : 18 : 18 : 18 : 18 : 20 : 20 : 22 : 22 : 35, distal sensory tufts on segments 3, 9 and 10. Palpal segments (Fig. 6, c) in proportion of 5 : 20 : 25 : 10 : 10, third segment swollen with large, shallow sensory pit.

Mesonotum (Fig. 6, b) dark brown with a pair of elongate, submedian, yellowish spots on disc and two sublateral pairs of irregularly rounded, bluish pruinose light spots; prescutellar area dark pruinose with the sensory areas darker; scutellum dark in middle. Postscutellum dark brown; pleura light brown, polished and darker across middle. Legs dark brown, femora with subapical, tibiae with subbasal, and hind tibiae with apical, narrow light rings.

Wing (Fig. 6, a) with radial cells complete, short, costa to 0.6 of wing length; macrotrichia scanty, a few only in cells R_5 and M_1. Large yellowish spots on anterior margin of wing at wing base and over r–m crossvein; the latter spot reaching slightly past anterior media into cell M_2. A broad dark area between these two spots and a very dark spot over second anterior radial cell and distal third of first. Cell R_5 with a reniform light spot just past end of costa and a large quadrate, oblique, subapical, pale spot with one side broadly attaining wing margin. Two pale spots in cell M_1, the second separated by a distance greater than its length from wing margin. Veins M_1 and M_2 pale bordered on at least distal halves. Cell M_2 with a small apical pale spot at wing margin and a long pale streak from base to level of pale spot in cell M_4, the latter spot covering distal half or more of cell; anal cell pale on basal third and two light spots at apex. Halteres pale.

Abdomen dark brown, spermathecae two, subequal, ovoid, the ducts slender and sclerotized a distance of about a fourth the length of spermathecae.

Male genitalia (Fig. 6 d, e) : Ninth sternite with low sublateral lobes and small mesal excavation, the posterior membrane bare; ninth tergite short, tapered, the apicolateral processes short, triangular and widely separated at bases. Basistyles with ventral roots boat-hook shaped, the dorsal roots not quite as long, stout and nearly straight; dististyles gently curved with slender, pointed apices. Aedeagus almost V-shaped, with anterior arch narrow and extending to about three-fourths of total length, distal portion slender with rounded apex and a pair of triangular, subapical lobes about half as long arising at level of the peak of the arch. Parameres (Fig. 6, d) with bases knobbed, stems gently curved and slightly swollen in mid-portions, rather abruptly narrowed past mid-lengths and abruptly recurved ventromesad with distal portions long and tapered to slender points with three long lateral barbs along subapical portions.

Holotype ♀, Pacora, Panama Prov., Panama, June 4, 1951, F. S. Blanton (light trap) (type No. 61499, U. S. N. M.). Allotype, Mojinga Swamp, Fort Sherman, Canal Zone, November 14, 1951, F. S. Blanton. Paratypes: 7 ♀♀, same data as allotype except August 28 to November 14; 3 ♀♀, Cerro Campaña, July 3, 1951; 3 ♀♀, Madden Dam, Canal Zone, September 21, 1951; 1 ♀, La Jolla, Panama Prov., October 5, 1951; 1 ♀, Lake Worth, Palm Beach Co., Florida, August 8, 1951, W. W. Wirth (bay shore).

This species resembles *galindoi* Wirth and Blanton in general facies and type of wing markings, but is readily distinguished by the yellowish submedian and bluish lateral mesonotal patches, the broadly pale margined veins M_1 and M_2 and the small round pale spot in cell M_1 separated by more than its length from wing margin. The Florida specimen, although far out of range from Panama, is included in the paratype series without hesitation as it agrees well in the characteristic mesonotal and wing markings.

SUMMARY

The following new species are described from material collected in light traps in Panama: *Culicoides hertigi, C. macrostigma, C. miyamotoi, C. gorgasi, C. mojingaensis,* and *C. tricoloratus.*

Explanation of Plate

Fig. 1. *Culicoides hertigi:* a, female wing; with important veins labelled; b, mesonotal pattern; c, female palpus; d, male genitalia; e, male parameres.

Fig. 2. *Culicoides macrostigma:* a, female wing; b, female palpus; c, male parameres; d, male genitalia.

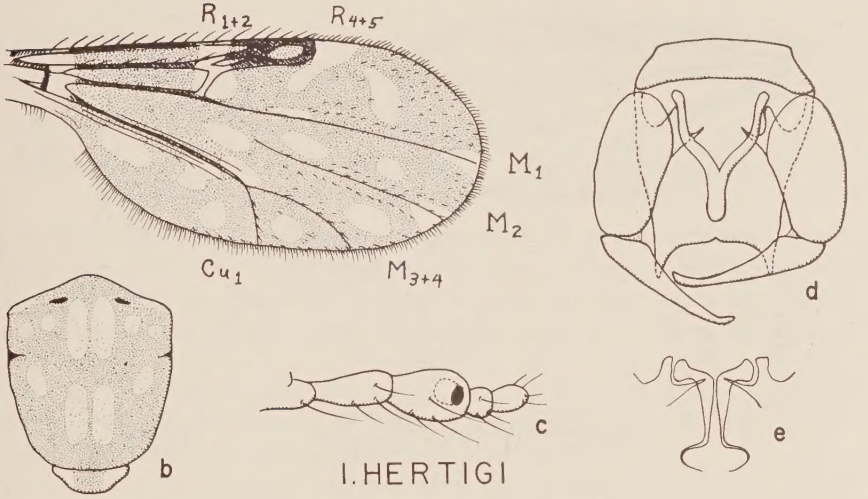
Fig. 3. *Culicoides miyamotoi:* a, female wing; b, mesonotal pattern; c, female palpus; d, male parameres; e, male genitalia.

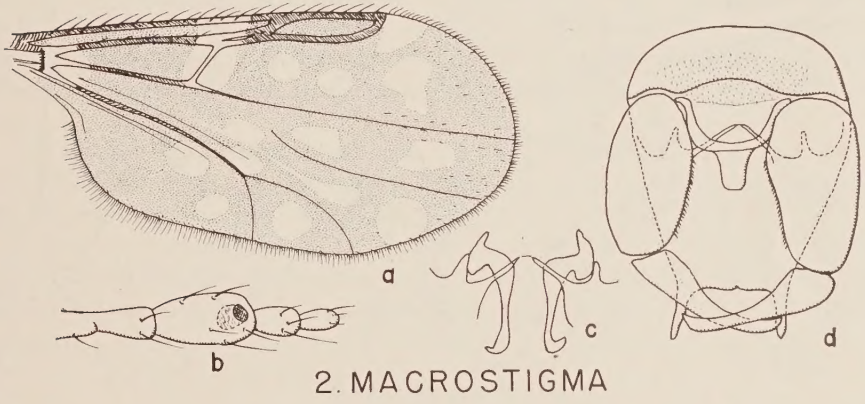
Fig. 4. *Culicoides gorgasi:* a, female wing; b, mesonotal pattern; c, female palpus; d, male parameres; e, male genitalia.

Fig. 5. *Culicoides mojingaensis:* a, female wing; b, mesonotal pattern; c, female palpus; d, female spermathecae; e, male parameres; f, male genitalia.

Fig. 6. *Culicoides tricoloratus:* a, female wing; b, mesonotal pattern; c, female palpus; d, male parameres; e, male genitalia.

PLATE I


R1+2
R4+5
M1
M2
Cu1
M3+4
b
c
d
e
1. HERTIGI


a
b
c
d
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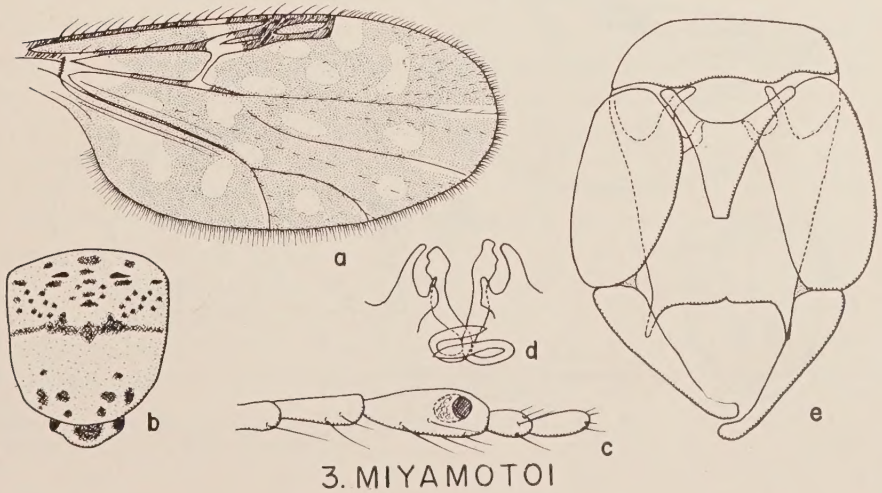
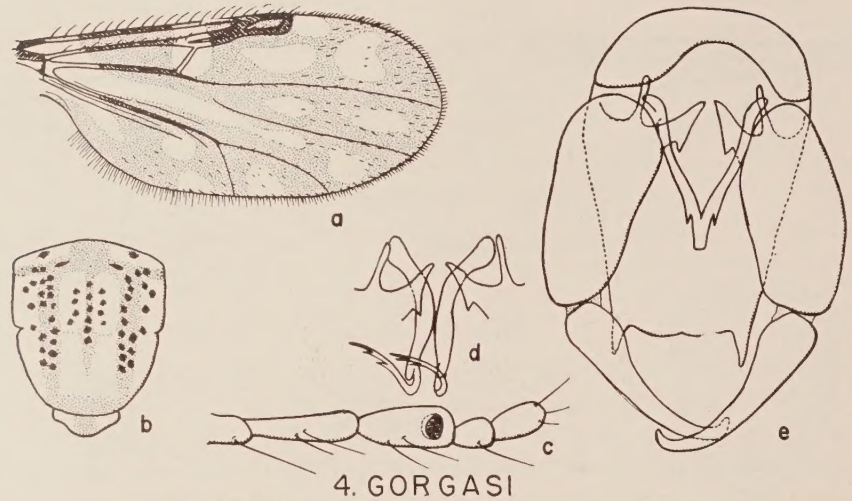
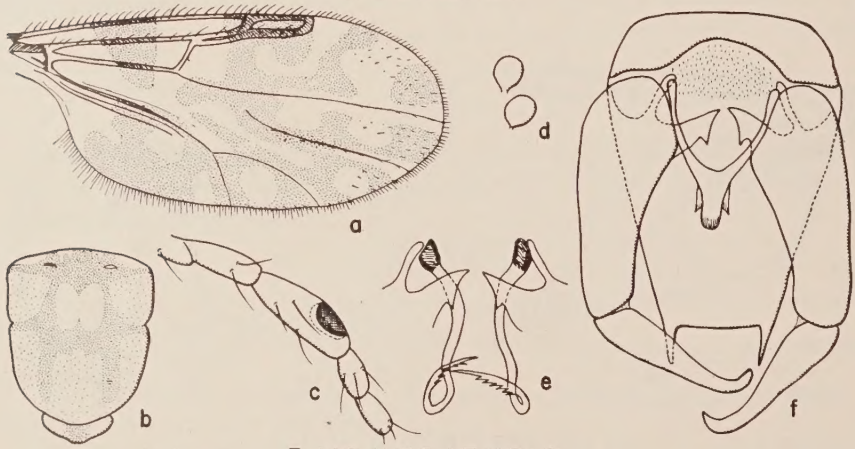

a
b
c
d
e
3. MIYAMOTOI

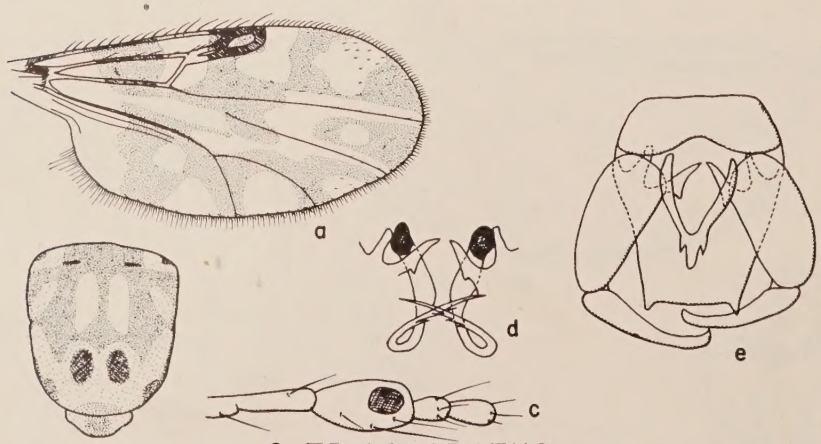
PLATE II



4. GORGASI



5. MOJINGAENSIS



6. TRICOLORATUS

OBSERVATIONS ON THE MIGRATION OF AVIAN SCHISTOSOMES IN MAMMALS PREVIOUSLY UNEXPOSED TO CERCARIAE

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The cercariae of schistosomes which normally reach the adult stage in birds have long been known to be able to penetrate the skin of man and various other mammals. When these cercariae penetrate man they may cause a severe papular dermatitis which has been the subject of extensive investigations over a period of many years (Cort, 1950).

Vogel (1930) and Brackett (1940) studied biopsies of typical papules caused by avian schistosomes in the human skin and found that the worms remained in the epidermis after penetrating and were rapidly destroyed there.

Recently, it was demonstrated experimentally (Macfarlane, 1949; Olivier, 1949) that the dermatitis induced in man by avian schistosomes is a sensitization phenomenon. It was shown that first exposure to the cercariae results in little or no skin reaction, but that repeated exposures usually are followed by the development of typical papular dermatitis. It was concluded that the papular dermatitis is a strong allergic response of the host which causes rapid destruction of the cercariae in the skin.

The absence of a reaction to the cercariae when they are put on the skin for the first time suggested that in unsensitized persons the cercariae might not stop in the skin but might migrate into other parts of the body.

In order to learn more about the capabilities of the avian schistosomes in regard to survival and migration in abnormal hosts, 5 species of laboratory animals were exposed to cercariae of 3 species of avian schistosomes and later examined for migrating worms. Some of the results of this study were presented earlier in abstract form (Olivier, 1949) and are presented here in more detail.

MATERIALS AND METHODS

The 3 avian schistosomes used were *Trichobilharzia stagnicolae* (*Cercaria stagnicolae*), *Trichobilharzia physellae* (*Cercaria physellae*), and *Trichobilharzia ocellata* (*Cercaria elvae*). All 3 species were shed by naturally infected snails collected in the vicinity of the University of Michigan Biological Station. Mice, hamsters, guinea pigs, rabbits, and monkeys were exposed to one or more of these three species by placing the cercariae on the clipped, moistened skin. All animals were given a single exposure to cercariae except that three of the rabbits were given two exposures one week apart. Some of the mice were killed with chloroform. However, most of the mice, including all those infected with *Trichobilharzia physellae*, were killed by injecting nembutal intraperitoneally. The monkeys were killed with nembutal given intravenously. Some of the hamsters were killed with chloroform; the remainder with nembutal injected intraperitoneally, as were all the guinea pigs.

Received for publication, July 2, 1952.

[1] Laboratory of Tropical Diseases.

Three of the rabbits were killed by injecting air intravenously while the other three were killed by injection of nembutal intraperitoneally.

Most of the autopsies were done within seven days after exposure. Skin, body wall, diaphragm, and other tissues were examined from some of the animals, but principal attention was paid to the lungs and liver since the former is the first large capillary barrier encountered by worms migrating from the skin by way of the blood or lymph, and the latter is the organ to which the worms would be destined to go if they were to survive and mature in the host.

The intact organs were first examined for lesions attributable to the parasites. The lungs and liver were then examined by various methods in an attempt to find other evidences of migration of the worms or to find the worms themselves. The lungs were examined by pressing them between glass plates, by teasing them apart under the dissecting microscope, or by teasing the lung tissue into small bits with needles, setting the teased material aside in physiological saline for one hour and then examining the sediment for worms (Olivier, 1952). Some of the livers were teased and examined under a dissecting microscope while others were perfused by the method of Yolles, et al. (1947).

OBSERVATIONS ON ANIMALS EXPOSED TO CERCARIAE OF *Trichobilharzia stagnicolae*[2]

Twenty-four white Swiss mice were exposed to from 500 to 900 cercariae of *Trichobilharzia stagnicolae* from *Stagnicola emarginata angulata* and autopsied from 1 to 8 days later.

Four of the mice had from 1 to 7 lung hemorrhages, but the liver, body wall, diaphragm, and exposed skin of the remaining 20 showed no evidence of worms or damage attributable to them. The lung lesions ranged in size from minute petechiae to hemorrhages 2 to 3 millimeters in diameter. Some of the hemorrhages were bright red with sharp margins, but in mice examined more than 4 days after exposure, some of the hemorrhages were deeper red and had diffuse and indistinct margins.

A portion of lung tissue from each of the mice was examined for living schistosomes. One worm was found in a lung hemorrhage of a mouse exposed 4 days previously and another in a lung hemorrhage of a mouse exposed 5 days previously. The 2 worms were readily identified as schistosomes since they had many of the characteristics of the body of schistosome cercariae. In one of the worms, the eye-spots were still intact while in the other the eye-spot pigment was present, but the granules were widely scattered (Plate 1, Fig. 8). Both worms had conspicuous, rounded gut ceca containing yellowish fluid in which were scattered small, brown, refractile granules.

The livers of all mice were teased under the dissecting microscope but no worms were found.

OBSERVATIONS ON ANIMALS EXPOSED TO CERCARIAE OF *Trichobilharzia ocellata*

All cercariae of *Trichobilharzia ocellata* used in these experiments were shed from specimens of *Lymnaea stagnalis.*

[2] Most of the experiments with *T. stagnicolae* in mice were done in cooperation with Dr. Paul P. Weinstein whose help is greatly appreciated.

Mice: Thirty white Swiss mice were exposed to cercariae of *T. ocellata.* Some of the data from these mice are presented in table 1.

TABLE 1.—*Data from mice exposed to cercariae of* Trichobilharzia ocellata

Interval between exposure and autopsy (days)	Number of mice examined after this interval	Approx. number of cercariae per mouse	Number of lung hemorrhages	Number of nodules on lungs	Number of worms from lungs
1	1	200	0	....	....
2	2	200, 300	18, many*	0, 0	2, 4
3	4	200, 200, 100, 100	14, 0, 2, 4	0, 0, 0, 0	0, 1, 3, 0
4	5	1000, 200, 300, 100, 200	many,* 15, many,* many,* 9	0, 0, 0, 0, 0	many,* .., 0, 4, ..
5	6	100, 200, ?, 500, 500	50, 4, 37, 3, few,* many*	0, 3, 0, 0 many,* many*	1, .., .., 1, 1, 1
6	1	300	3	50	....
7	4	300, 300, 100, 500	1, 2, 0, 1	64, 43, 20, few*	....
8	1	500	0	many*	....
9	2	100, 200	7, 5	8, 1	....
11	2	200, 500	0, 1	1, 20	....
12	1	?	0	8	....
16	1	?	1	1	....

* Exact count not recorded.

Hemorrhages essentially identical with those seen in mice exposed to cercariae of *T. stagnicolae* were observed on the lungs of 25 of the mice, and in at least 5, the lung hemorrhages were very numerous. The number of hemorrhages was usually larger in the mice examined from 1 to 6 days after exposure than in those examined later. Most of them were distinct, discrete, and apparently distributed at random over the pleural surfaces of the lungs. Lung dissections revealed a small number of internal hemorrhages not apparent from the outside.

Small, colorless, nodular lesions from approximately 0.5 to 2.0 mm. in diameter were observed on the lung surfaces of almost all mice examined more than 5 days after exposure. The nodules were most numerous from the fifth to the eighth day after exposure.

The lungs of 13 mice were examined for worms, and in 10 one or more worms were found. All the worms could readily be identified as schistosomes (Plate 1, Fig. 6). Some had intact eye-spots while in others the eye-spot granules had become dispersed. In almost all worms the gut was larger and longer than that of the cercariae, and in some the ceca had united posterior to the acetabulum. Usually the gut contained numerous brown, refractile granules, but some contained only pale fluid. The worms ranged in length from 0.20 to 0.37 mm.

Numerous small schistosomes were found in sections of lung from one mouse and 2 worms were found in sections of a small portion of lung from another mouse.

The liver of 19 of the mice were perfused but no worms were found.

Hamsters. Each of 12 Syrian hamsters was exposed to from 50 to 500 cercariae of *Trichobilharzia ocellata.* The animals were autopsied 4, 5, 6, 7, and 11 days later.

Lung hemorrhages, usually few in number, were observed in 9 of the 12 hamsters (Plate 3, Fig. 1). Small colorless nodules similar to those described from the lungs of mice were noted on the lungs of all hamsters examined more than 4 days after exposure (Plate 3, Figs. 2 and 3).

Part of the lung from each of 8 hamsters was examined for worms, and 1 or more worms were found in 3. All but one worm resembled the body of the cercaria, and they ranged in length from 0.20 to 0.22 mm. The gut was much larger than

that of the cercariae and usually contained a dense accumulation of small, brown, refractile granules. (Plate 1, Fig. 4.)

One worm, recovered from a large hemorrhagic lesion on the lung surface of a hamster exposed 7 days previously was larger than the others (0.37 mm. long) and had the body proportions of an adult (Plate 1, Fig. 7). Its oral sucker resembled that of the mature worm, and the gut was very large, was full of refractile granules, and had the gross appearance of that in the adult.

Lung tissue from 3 hamsters was sectioned. The lung of one hamster had worms associated with hemorrhages and areas of cellular infiltration. (Plate 2, Fig. 4.) The second hamster had many large areas of cellular infiltration and worms apparently moribund or dead, associated with these lesions. The third had large nodular lesions but no worms.

Guinea pigs. Eight guinea pigs were exposed to either 50 or 200 cercariae of *T. ocellata* and examined 6 days later.

From 2 to 7 hemorrhages were found on the lungs of 4 animals exposed to 50 cercariae and from 4 to 14 hemorrhages were found on the lungs of the 4 animals exposed to 200 cercariae. The 4 animals exposed to 50 cercariae had 1, 2, 3, and 18 small, colorless nodules on the lung surfaces while the 4 animals exposed to 200 cercariae had 0, 5, 6, and 10 nodules.

The lungs and livers of the guinea pigs were not examined for worms.

Rabbits. Two rabbits were given a single exposure to the cercariae and examined 2 and 10 days later after the animals were killed with nembutal. One was given a single exposure, killed 4 days later by air embolism, and examined. The lungs of the rabbits killed 2 and 4 days after exposure had numerous, bright red, distinct hemorrhages. The one killed on the tenth day had a few bright red hemorrhages but many were large and dark and others were apparently in various stages of resolution. In addition, the lungs of this rabbit had numerous small nodular lesions ranging from pink to colorless and about the same size as the dark red hemorrhages. Three rabbits were given two exposures one week apart and examined 15, 26 and 43 days after the last exposure. Two were killed by air embolism while the other was killed with nembutal. No hemorrhages were found on the lungs of these rabbits but all 3 had numerous small colorless nodules such as were seen on the lungs of the rabbit with the 10-day infection.

It should be noted that although 2 of the last 3 rabbits were killed by air embolism they had no petechial hemorrhages thus indicating that this technique was probably not the cause of the pulmonary hemorrhages encountered in the rabbit examined 4 days after exposure.

Monkeys. Each of 2 rhesus monkeys was exposed to several hundred cercariae of *T. ocellata* and examined 4 days later. Another was exposed to several hundred cercariae and autopsied 12 days later. Forty-four small hemorrhages were seen on the lung of one of the monkeys (No. 1) examined after 4 days, and 8 hemorrhages from 2 to 3 mm. in diameter were found on the lung of the other. Twelve large (2 to 3 mm.) hemorrhages and 10 colorless nodules were found on the lung of the third monkey (Plate 3, Fig. 6).

Small portions of lung from all 3 monkeys were examined for worms, and as a result 2 living worms were recovered from monkey No. 1. Each worm was about 0.20 mm. long and resembled the body of the cercariae (Plate 1, Figs. 2 and 3).

Two small bits of lung tissue from the same monkey were sectioned, and one worm was found in each.

OBSERVATIONS ON MICE EXPOSED TO CERCARIAE OF *Trichobilharzia physellae*

A total of 33 white Swiss mice were exposed to cercariae of *Trichobilharzia physellae* from *Physa parkeri.* Twelve mice were exposed to from 200 to 800 cercariae in small groups and autopsied from 3 to 11 days later. All eight mice examined from 3 to 7 days after exposure had lung hemorrhages while only one of the four examined 8 to 11 days after exposure had hemorrhages. The lungs of four mice examined 3 and 4 days after exposure had no colorless nodules while all the mice examined more than 4 days after exposure had numerous colorless nodules. Worms were recovered from the lungs of three of the four mice examined 3 and 4 days after exposure. Twenty-one mice constituted a single experiment in which all were exposed individually to 225 cercariae from the same source and autopsied at intervals from 2 to 19 days after exposure in an attempt to gather comparable data on the number of hemorrhages, nodules, and worms present at various periods after exposure (table 2). In this experiment the lungs of all mice were examined grossly for hemorrhages and nodules, and all except 8 were examined for worms by teasing in saline; these 8 were fixed for sectioning.

TABLE 2.—*Data from 21 mice exposed simultaneously to 225 cercariae of* Trichobilharzia physellae

Interval between exposure and autopsy (days)	Number of mice examined after this interval	Number of lung hemorrhages	Number of nodules on lungs	Number of worms from lungs
2	3	2, 2, 0	0, 0, 0	. ., 0, 0
3	3	6, 25, 5	0, 0, 0	1, . ., 1
4	3	26, 16, 11	0, 0, 0	. ., 0, 1
5	3	16, 5, 3	5, 19, 5	. ., 0, 0
6	3	3, 7, 3	8, 14, 7	0, 0, 0
9	3	1, 2, 0	9, 19, 4	0, 0, . .
12	1	0	20	. .
15	1	0	3	. .
19	1	1	5	. .

Hemorrhages were seen on the lungs of almost all the mice exposed to *T. physellae.* Two days after exposure, the mice had few hemorrhages; the number of hemorrhages was greatest on the third, fourth, and fifth days and very few hemorrhages were seen after the fifth day.

Colorless nodules were found on the lungs of all mice examined more than 4 days after exposure, but they were most numerous from the fifth to the twelfth days after exposure.

Perfusion of the livers from all 33 mice revealed no worms.

DISCUSSION

When mice, hamsters, guinea pigs, rabbits, and rhesus monkeys were exposed to avian schistosome cercariae, almost all of the animals provided either direct or indirect evidence that the worms penetrate and migrate to the lungs.

Direct evidence of penetration and migration came from the recovery of living worms from the lungs. Migrating individuals of all 3 avian schistosome species were found in the lungs in sufficient numbers so that their recovery there cannot be

considered exceptional. None of the worms from the lungs had grown appreciably in size, and only one (Plate 1, Fig. 7) had undergone extensive morphological change. However, the gut was much larger and more conspicuous than that of the cercariae and usually contained brown refractile granules very similar to those seen in the migrating forms of *Schistosomatium douthitti* (Olivier, 1952). The granular material probably represented breakdown products of ingested blood.

It is noteworthy that even though the bird schistosomes migrating in laboratory animals apparently ingest blood, as shown by the appearance in the gut ceca of pigment probably derived from blood, the worms did not grow and were not found in the lungs more than 5 days after exposure. No schistosomes were found in the liver.

It is concluded for the following reasons that the lung hemorrhages were a consequence of the migration of the schistosomes to the lungs and may be taken as indirect evidence of such migration: (1) Hemorrhages were not seen on the lungs of normal animals not exposed to schistosomes either in these experiments or in a previous study using mice (Olivier, 1952), (2) the hemorrhages were found on the lungs of almost all the experimental animals autopsied within seven days after exposure when migration would probably occur if it were to occur at all, (3) they were not found in most animals autopsied more than 7 days after exposure, (4) living schistosomes from the test exposures were found in the lungs at the time the hemorrhages were most numerous, (5) many of the living worms recovered were taken from hemorrhages, and worms were found associated with hemorrhages in sectioned material, and (6) the hemorrhages were closely similar to those found on the lungs of mice exposed to *Schistosoma mansoni, Schistosoma japonicum* and *Schistosomatium douthitti* and attributed to migrating worms (Olivier, 1952).

The small colorless nodules seen on the lungs of many of the animals exposed to the bird schistosomes are interpreted to be areas of resolution of hemorrhage and areas of cellular response to dead or dying schistosomes. The nodules appeared on the lungs closely following the peak of lung hemorrhage and thereafter disappeared rapidly. Moreover, sectioned nodules from a hamster lung contained schistosomes which were either moribund or dead.

It is clear that bird schistosomes which cause dermatitis in man can penetrate the skin of unsensitized laboratory animals and migrate to the lungs. The fate of these species of schistosomes in unsensitized humans is unknown. However, in view of the experience with the cercaria of *T. ocellata* in rhesus monkeys, it seems possible that exposure of an unsensitized human to bird schistosomes may result in the migration of worms to the lungs and production of petechial hemorrhages there.

It should be noted in this connection that *Schistosomatium douthitti,* a parasite of small mammals in the United States and a known cause of schistosome dermatitis in man, may migrate to the lungs of the rhesus monkey in large numbers (Penner, 1941). This species, perhaps, has a greater potentiality for causing significant lung lesions in man than have the bird schistosomes.

SUMMARY

Cercariae of *Trichobilharzia stagnicolae, Trichobilharzia physellae,* and *Trichobilharzia ocellata,* all schistosomes of birds, penetrated the skin, migrated to the lungs, and produced pulmonary hemorrhages in laboratory mice. The migration of

cercariae of *T. ocellata* was also studied in hamsters, guinea pigs, rabbits, and rhesus monkeys, and pulmonary hemorrhages were also found in these animals. Worms of all 3 species were recovered from the lungs of one or more of the 5 host species, but none was recovered from the liver.

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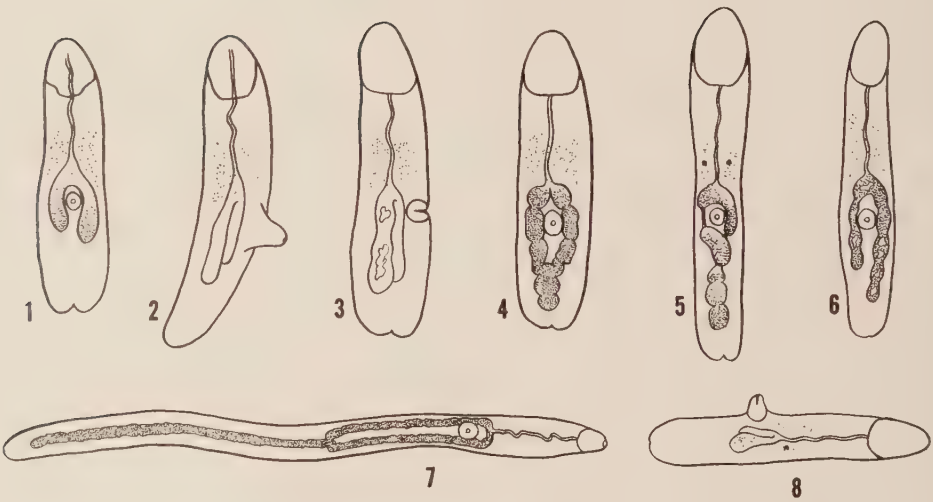
PLATE I



All drawings are to the same scale.

FIG. 1. Worm from the lung of a mouse exposed 4 days previously to *Trichobilharzia physellae*. Note many refractile granules in the gut and scattered eye-spot pigment granules.

FIGS. 2 and 3. Worms from the lung of a rhesus monkey exposed 4 days previously to *Trichobilharzia ocellata*. Gut ceca empty.

FIG. 4. Worm from the lung of a hamster exposed 4 days previously to *Trichobilharzia ocellata*. Many refractile granules in gut.

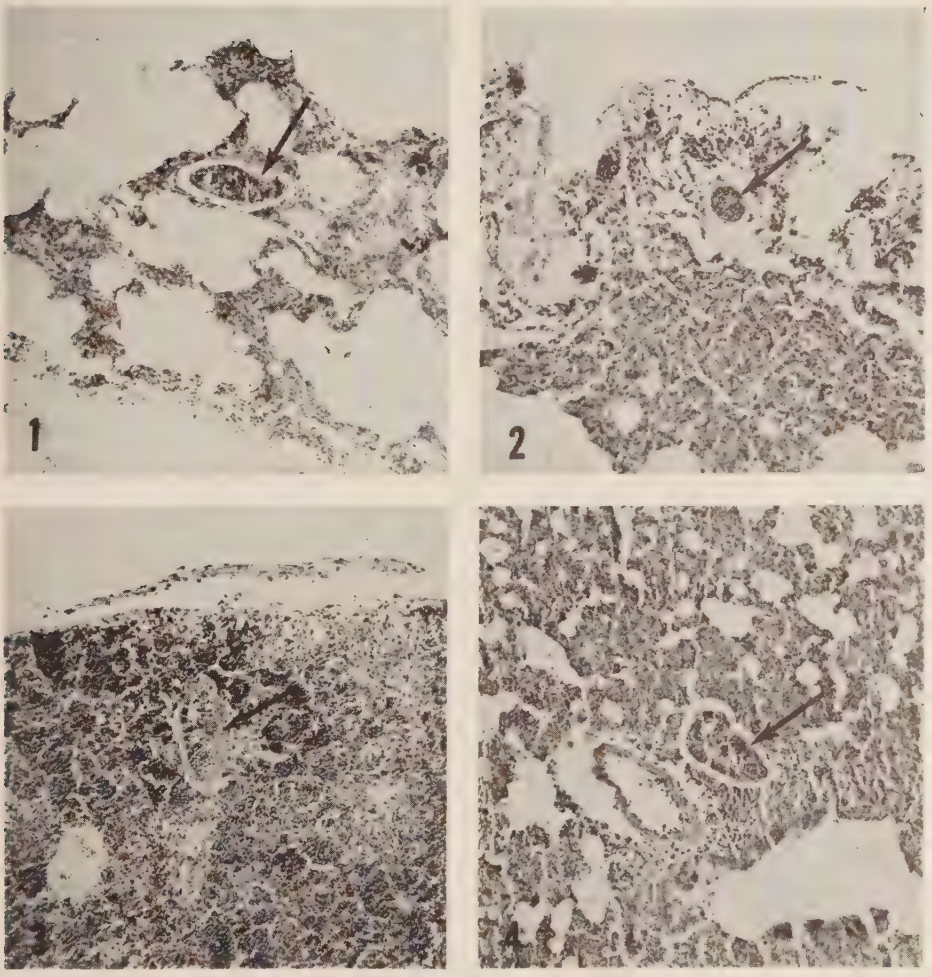
FIG. 5. Worm from the lung of a rabbit exposed 4 days previously to *Trichobilharzia ocellata*. Many granules in the gut. Eye-spots only partly dispersed.

FIG. 6. Worm from the lung of a mouse exposed 5 days previously to *Trichobilharzia ocellata*. Many granules in the gut.

FIG. 7. Worm from the lung of a hamster exposed 7 days previously to *Trichobilharzia ocellata*. Note extensive change toward adult structure despite small size.

FIG. 8. Worm from the lung of a mouse exposed 5 days previously to *Trichobilharzia stagnicolae*. Few refractile granules in the gut.

PLATE II

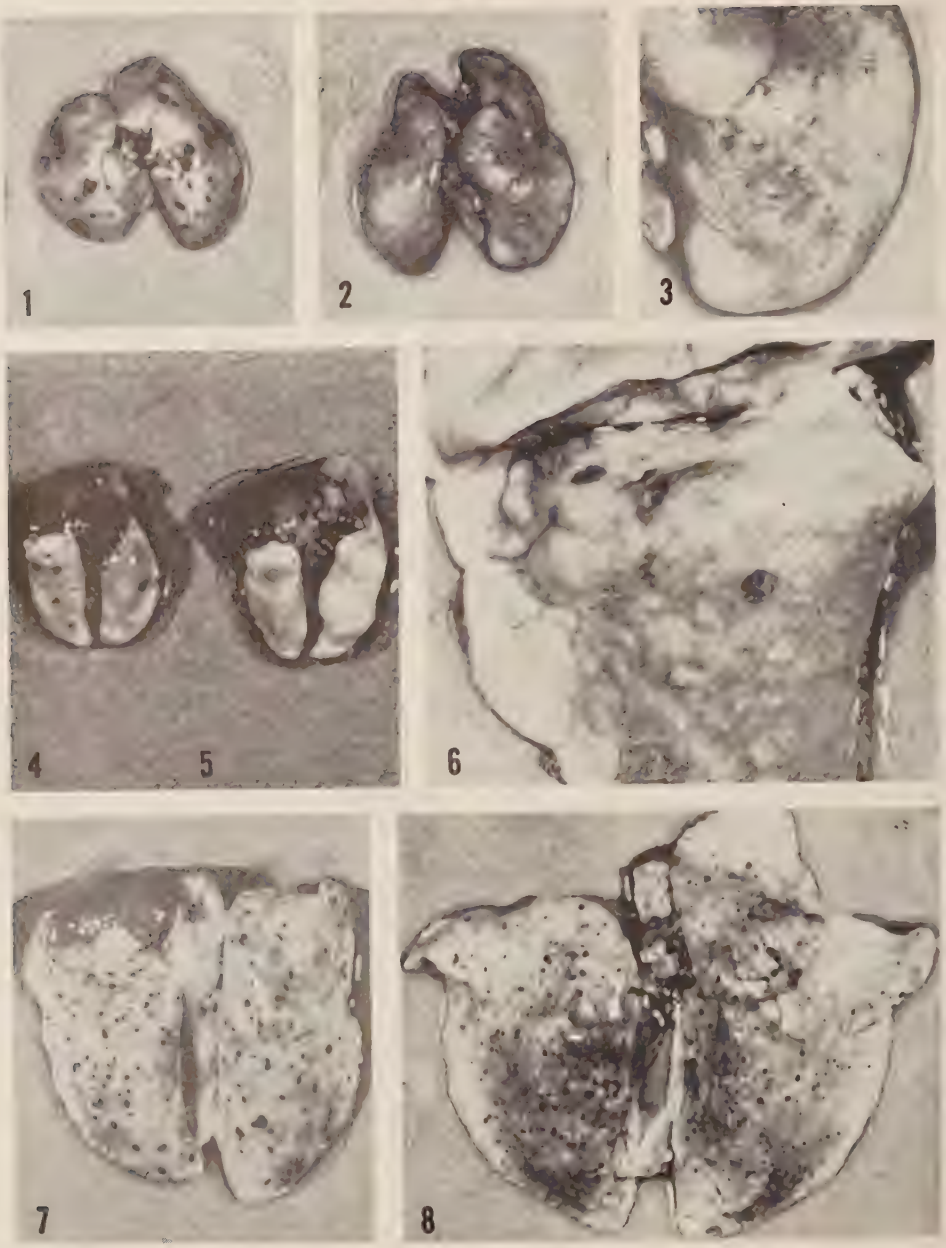


Photographs of *Trichobilharzia ocellata* in sections of lungs from laboratory animals.

FIGS. 1. and 2. Worms in the lung of a rhesus monkey exposed 4 days before autopsy.

FIG. 3. Worm in the lung of a white Swiss mouse exposed 4 days before autopsy. There was massive hemorrhage in the lung.

FIG. 4. Worm in the lung of a hamster exposed 4 days before autopsy. The worm was surrounded by a large cellular reaction.



Lesions on the lungs of laboratory animals exposed to cercaria of *Trichobilharzia ocellata.*

FIG. 1. Lung of a hamster exposed 4 days previously to about 100 cercariae. Numerous large hemorrhages, some of them confluent, are apparent.

FIG. 2. Lung of hamster exposed 10 days previously to about 500 cercariae. No hemorrhages are present but numerous small nodules may be seen.

FIG. 3. Enlarged portion of figure 2 showing nodular lesions.

FIG. 4. Lung of a mouse exposed 4 days previously to 200 cercariae.

FIG. 5. Lung of a normal mouse.

FIG. 6. Lung of a rhesus monkey exposed 12 days previously to about 500 cercariae. Three large hemorrhages, probably attributable to the parasites, are visible.

FIG. 7. Lung of a rabbit exposed 2 days previously to 300 cercariae. Only bright, distinct hemorrhages are visible.

FIG. 8. Lung of a rabbit exposed 10 days previously to about 500 cercariae. A few new hemorrhages, many old hemorrhages, and many nodular lesions are visible.

TROMBICULA (TROMBICULINDUS) KANSAI, A NEW CHIGGER FROM CENTRAL HONSHU, JAPAN[1]

E. W. Jameson, Jr.[2] and Manabu Sasa[3]

The subgenus *Trombiculindus* was established as a genus by Radford (1948) for a species of chigger taken from rats (*Rattus* sp.) in India. Subsequently, Traub and Evans (1951) described two more species of *Trombiculindus* from shrews (*Crocidura* sp. and *Suncus* sp.) from North Burma. Recently, Womersley (1952, p. 140) named a fourth species of this group; his species was based on specimens found on an unidentified mouse taken in Bal Tal Kashmir, India. The chigger here described is the fifth member of *Trombiculindus;* but, unlike the previously named species, it occurs in temperate regions, far to the north of the known geographic ranges of the other species. We prefer to follow Wharton et al (1951) in using *Trombiculindus* as a subgenus of *Trombicula.* Although the foliate setae are strikingly different from the slender, feathered setae of most other species of *Trombicula,* such a character by itself seems not to be sufficient for the separation of this group as a genus. Except for the foliate setae, these species have the characters of the subgenus *Leptotrombidium.*

Trombicula (Trombiculindus) kansai, new species
(Fig. 1)

Most of the specimens were in an apparently unengorged condition; the shape is rather terete and the color is pale yellow. Mounted specimens are somewhat depressed and appear broader than in life.

Gnathosoma: Chelicera with a subapical dorsal tooth; cheliceral base with numerous, small punctae. Capitular sternum with a pair of feathered setae and numerous, small punctae. Palpal femur and palpal genu each with a nude seta; dorsal tibial seta feathered, and lateral and ventral tibial setae nude. Palpal claw three-pronged. Palpal tarsus (thumb) with seven feathered setae and a small, slender spur (its length about equal to the width of the tarsus). Galeal seta feathered.

Legs: All coxae unisetose; the seta on coxa III distinctly behind the anterior margin of the coxa. Specialized (nude) setae: Leg I, 2 genualae, 1 microgenuala, 2 tibialae, 1 microtibiala, 1 spur, 1 microspur, 1 subterminala, 1 parasubterminala, 1 pretarsala; Leg II, 1 genuala, 2 tibialae, 1 spur, 1 pretarsala; Leg III, 1 genuala, 1 tibiala. Each tarsus with two claws and a claw-like empodium.

Scutum: More or less rectangular, more than twice as broad as long, with rounded posterior corners and a straight posterior margin. Punctae numerous and delicate. Anterolateral setae slender and densely feathered; anteromedian seta with a longer and heavier shaft and fewer branches. Posterolateral setae foliaceous, about three and one-half times as long as wide, with small barbs on the margins and on the surface. Sensillary bases slightly behind a line connecting posterolateral setae. Basal third of sensillae nude; remainder with 14 to 18 branches. Scutal measurements of holotype (in microns): AW-70, PW-82, SB-33, ASB-29, PSB-17, AP-20, AM-79, AL-44, PL-58, S-64.

Setae: Setae of dorsum similar to posterolateral scutal setae, the lateral ones wider. Two pairs of humeral setae (DS_1). Dorsal setal formula: 4-10-12-12-10-4. Anterior ventral setae mostly slender and feathered; two pairs of sternal setae and about twenty additional feathered setae caudad from the coxae. Sixteen to eighteen foliate setae on venter.

Received for publication, August 29, 1952.

[1] Studies upon which this paper is in part based were conducted under contract no. DA-49-007-MD-242, between the University of California and the Department of the Army.

[2] Far East Medical Research Unit, U. S. Army, on leave from the University of California, Davis, California.

[3] Institute for Infectious Diseases, University of Tokyo, Japan.

Types: Holotype from the shrew-mole, *Urotrichus talpoides* Temminck and Schlegel (Talpidae), 8 March 1952, Ohara Area (near Kyoto City, Kyoto Prefecture, Honshu, Japan). Twenty-four paratypes from *Urotrichus talpoides* from Kyoto, Mie, and Shiga Prefectures, collected from March to June, 1952. Holotype and ten paratypes deposited in the United States National Museum. Paratypes deposited with the following institutions: Rocky Mountain Lab-

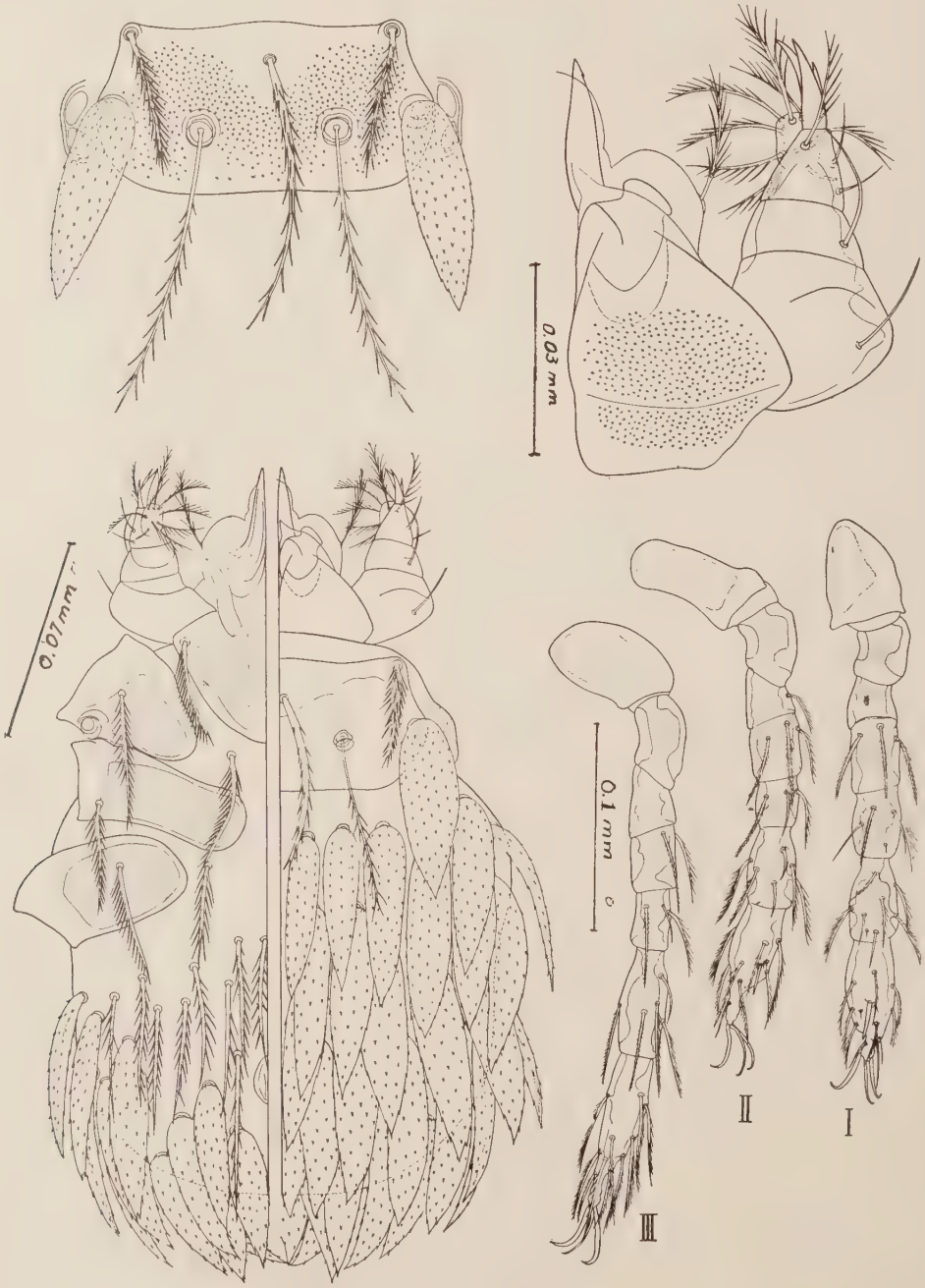


Fig. 1. *Trombicula kansai* n. sp.

oratory (Hamilton, Montana), British Museum (Natural History) (London), Institute for Infectious Diseases, University of Tokyo (Tokyo), Kitasato Institute (Tokyo), Department of Parasitology, Kyushu University (Fukuoka), and the South Australian Museum (Adelaide).

Key to Larvae of the Subgenus *Trombiculindus*

1. All ventral setae feathered 2
   With some ventral setae foliate 3
2. PL about three times as long as wide, with small barbs; sensillae with basal barbs *cuneatus* Traub and Evans
   PL less than two times as long as wide, with reticulations; sensillae nude basally *foliaceus* Traub and Evans
3. PL less than two times as long as wide, with punctae ..... *squamosus* Radford
   PL about three to three and one-half times as long, with small barbs 4
4. With one pair of humeral setae; four to six foliate setae on venter .. *squamifera* Womersley
   With two pairs of humeral setae; sixteen to eighteen foliate setae on venter ... *kansai*, n. sp.

This chigger is very common on shrew-moles in Kyoto Prefecture and has been found less commonly in nearby areas. In addition to our collections, it was found at Uji in Kyoto Prefecture by Dr. H. Kamo of the Department of Parasitology, Kyushu University Medical School. Extensive collecting by the junior author indicates that it is rare or unknown in many other parts of Japan. *Trombicula kansai* frequently attaches at the base of the tail of the host, and sometimes 100 or more chiggers may occur on a single host. A few specimens of this chigger were taken from mice (*Apodemus geisha* and *A. speciosus*). "Kansai" is the Japanese name for the region in which this mite occurs most commonly.

The illustrations were made by Mr. Akira Shimazoe.

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A CATERPILLAR (LEPIDOPTERA: PHALAENIDAE) FROM THE DIGESTIVE TRACT OF A HUMAN

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On June 4, 1952 in London, Ontario, a woman fifty-two years of age passed with the feces a caterpillar 16 mm. long (Fig. 1). To the physician investigating

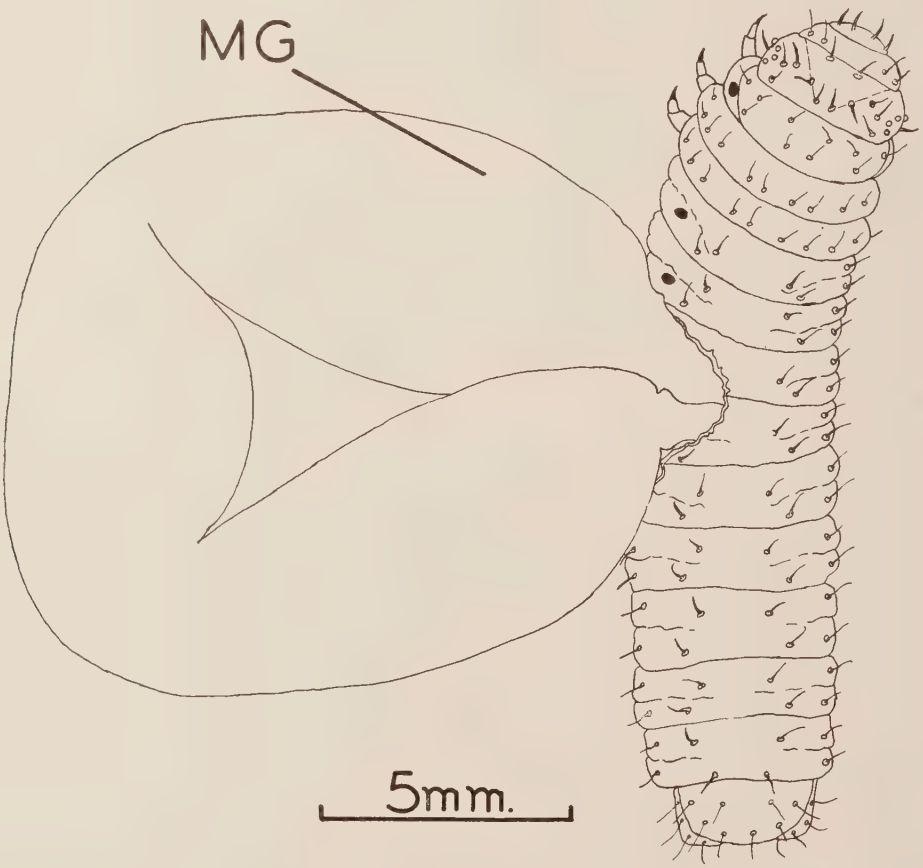

Fig. 1. Dorsal view of larva with mid-gut (MG) extending laterally through the ruptured integument.

the case she reported having experienced abdominal distress for a few days previously. Upon examination the caterpillar proved to be a larva of the family Phalaenidae as identified in Peterson's key (1948). The integument was unaffected and its setae remained intact so the details of the chaetotaxy and the crochets on the plantae could be used in identifying the specimen. It keyed out in a couplet including the "smooth" cutworms. At the left side of the third and fourth abdominal

Received for publication, August 7, 1952.

segments the integument was ruptured and the mid-gut of the larva extended laterally out of the opening in a broad loop (Fig. 1-MG). This extrusion of the digestive tract had allowed a telescoping of the body segments, so the length of the larva, in its intact state, was probably considerably greater than 16 mm. The loop of mid-gut was opened and was found to be crammed with green, undigested plant tissue. The likelihood is that the larva had been ingested with green vegetable food and during its passage through the alimentary canal of the patient the integument had been broken with consequent extrusion of the digestive tract.

In discussing the ways in which insects attack animals, Kirby and Spence (1818, 1826) used the term *scolechiasis* to denote the invasion of animal tissues by larval insects. They cited an instance of a boy vomiting up caterpillars. Hope (1840) restricted the term (*scholechiasis*) to cases involving lepidopterous larvae only, as distinct from cases involving coleopterous larvae (*cantharíasis*) and dipterous larvae (*myiasis*) and he gave various examples of the occurrence of caterpillars in humans, e.g. larvae of *Noctua*. Matheson (1950) also restricted the term (*scoleciasis*) to instances involving lepidopterous larvae and stated that no reports of its occurrence appear in recent literature. It is evident from examples cited by these authors that well authenticated cases of scoleciasis of human tissues are rare and that instances of invasion by caterpillars are mostly accidental. In the case reported by the present writer the caterpillar was probably accidentally ingested with the food.

The writer is indebted to Dr. G. R. Bourne, M.D. of London for supplying information regarding the case and to Dr. W. M. Wilson, M.D. of the Regional Laboratory, Ontario Department of Health, London for submitting the specimen for examination.

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PERUTAENIA THRELKELDI, N. G., N. SP. (CESTODA: ANOPLOCEPHALIDAE) FROM *LAGIDIUM PERUANUM**

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Five specimens of *Lagidium peruanum* Meyen, a small rodent which lives in the high Sierra of Peru, were collected during the years 1950 and 1951. The five rodents came from the province of Jauja, at an altitude of approximately 11,900 feet.

On opening the intestine many very small cestodes of the subfamily Anoplocephalinae Blanchard, 1891, were discovered. A review of the available literature indicates the present material represents a new genus for which the name of *Perutaenia* is proposed. The specific name, *P. threlkeldi,* was proposed in honor of Dr. William Logan Threlkeld.

MATERIALS AND METHODS

A total of sixty-two specimens of the new anoplocephalid was fixed in 10% formalin. Fifty-seven of the cestodes were mounted *in toto* after being stained in Delafield's hematoxylin, ferric acetocarmine and creosote-azo-carmine mixture. Some of these were cleared in xylene while others were cleared in creosote. Five specimens were sectioned in series; two transversely, two longitudinally, and one sagittally. The sectioned material was stained with Delafield's hematoxylin and eosin.

Perutaenia n. g.

Diagnosis: Anoplocephalinae: Small-sized cestodes with linear segmentation. Genital apertures regularly alternate. Sexual ducts passing dorsally to osmoregulatory vessels and nerve trunks. Testes few in number, placed anteromedianly in segment, and equally dispersed on both sides of the midline. Cirrus pouch well-developed. Cirrus spiny. Female glands situated in the middle and posterior part of the segment. Uterus a transverse and lobulated sac between the osmoregulatory vessels. Eggs with a well-developed pyriform body. Adults in rodents.

Type species: Perutaenia threlkeldi (Parra, 1952).

Perutaenia threlkeldi (Parra, 1952)

(Figures 1–7)

Diagnosis: This is a very small cestode consisting of 25 to 41 proglottids having serrated margins. The length ranges from 6 mm. to 14 mm. while the width varies from 0.40 to 2.00 mm., with the maximum width attained near the middle of the strobila. Behind the scolex the first segment measures 0.03 to 0.05 mm. in length by 0.34 to 0.60 mm. in width. The matured segment measures 0.20 to 0.33 mm. in length and 0.91 to 1.67 mm. in width. The gravid segments

Received for publication, August 7, 1952.

* Appreciation is expressed here to Doctors W. L. Threlkeld and I. D. Wilson for their aid, supervision, and encouragement during the course of this work and to Dr. M. O. Veliz for the material which made this study possible.

The writer gave a short account of this parasite at the Thirteenth Annual Meeting of the Association of Southeastern Biologists, Agnes Scott College, Decatur, Georgia, April 18, 1952, in which the form was placed in the genus *Paranoplocephala.* Further study and review of the literature clearly indicate that it does not belong in this genus but should be regarded as representing a new genus.

A part of a thesis submitted to the graduate faculty of the Virginia Polytechnic Institute in partial fulfillment of the requirements for the Master's degree.

measure 0.60 to 1.13 mm. in length and 0.64 to 1.46 mm. in width. Numerous calcareous corpuscles were observed in the strobila The width of the unarmed scolex exceeds that of its length: it measures 0.33 to 0.47 mm. in length by 0.44 to 0.53 mm. in width. The circular suckers measure 0.16 to 0.19 mm. in diameter. There is no neck and segmentation begins immediately behind the scolex. Four longitudinal osmoregulatory vessels are present; two running along each lateral margin of the segment, ventrally to the cirrus pouch. The ventral osmoregulatory vessel is 21 to 24 microns in diameter and the dorsal is 3 to 6 microns in diameter. The latter vessels are situated laterally to the ventral ones. Genital primordia appear in the first segment. The genital atria regularly alternate, are comparatively large and are deeply situated in the mid-level of the proglottid or slightly posterior to the level. In a mature proglottid an atrium may be indented as much as 0.17 mm. and may measure 0.08 to 0.09 mm. in diameter at the margin, although its width may increase to as much as 0.17 mm. internally (figures 1, 3, 5, and 6). Fifteen to twenty small round testes, about 0.03 mm. in diameter, are present: they lie mostly anterior to the female glands, close to the dorsal surface, and are confined to the space between the osmoregulatory vessels although about equally divided by the mid-line. The cirrus pouch is large (0.36 to 0.44 mm. long by 0.13 to 0.16 mm. wide), possesses a strong muscular wall composed of longitudinal and transverse muscles, and extends inward well beyond the osmoregulatory canals, through about one-third of the width of a segment. Its position is horizontal in the mature segment and diagonal in the gravid segment. Both an external and an internal seminal vesicle are present. The cirrus 0.33 mm. long by 0.03 mm. wide, is armed with spines. The female glands are placed ventrally in the middle and posterior part of the segment. The small ovary forms two oval lobules, with each lobule measuring about 0.08 by 0.06 mm. Initially the uterus is a transverse elongated sac which later develops into a lobulated structure: it is confined to the area between the osmoregulatory canals, never extending beyond them even in the gravid segments. The vagina was observed in longitudinal sections only; it is very inconspicuous and runs parallel to the cirrus pouch, anterior to the middle of its ventral face. The seminal receptacle was not observed. The eggs possess a well-developed pyriform body which has two horns. The diameter of the egg measures 60 to 66 microns and the onchosphere is about 12 microns in diameter.

Host: Lagidium peruanum Meyen.
Location: Small intestine.
Geographic distribution: Peru, South America.
Type specimen: U. S. Nat. Mus. Helminth. Coll., No. 37,380.

DISCUSSION

According to Baer (1927) and Wardle and McLeod (1952) the following seven genera of the subfamily ANOPLOCEPHALINAE have been reported from rodents: *Cittotaenia* Riehm, 1881, *Fuhrmannella* Baer, 1925, *Andrya* Railliet, 1893, *Diandrya* Darrah, 1930, *Bertiella* Stiles and Hassall, 1902, *Paranoplocephala* Lühe, 1910, *Monoecocestus* Beddard, 1914, and *Prototaenia* Baer, 1927. *Parabertiella* Nybelin, 1917, although reported from a marsupial, is included in the comparisons made in this paper because it shows a well-developed cirrus pouch and a single set of reproductive organs. *Cittotaenia, Fuhrmannella,* and *Diandrya* differ from *Perutaenia* in having a double set of reproductive organs. *Andrya* differs from *Perutaenia* in that the genital pores are situated unilaterally or irregularly alternate; the testes are on the aporal side of the segment; the cirrus pouch is small; the female genitalia are on the poral side; and the uterus is typically reticular. *Bertiella* differs from *Perutaenia* in that the genital pores are irregularly alternate; the cirrus pouch is poorly developed; and the female glands are in the middle of the poral side of the segment. *Paranoplocephala* differs from *Perutaenia* in that the genital pores are unilateral or irregularly alternate; the testes are numerous, and situated aporally to the ovaries and often extend beyond the osmoregulatory canal on that side; the female glands are situated in the middle of the poral side of the segment; and the uterus is a transverse tube and extends beyond the osmoregulatory canal and towards the ventral face. *Monoecocestus* differs from *Perutaenia* in that the osmoregulatory canals are characterized by numerous anastomoses; the testes are

situated in the middle of the posterior part of the segment; the vagina is placed anterior to the cirrus pouch; and the uterus is reticular. *Prototaenia* differs from *Perutaenia* in that it is characterized by having irregularly located genital pores; there are numerous testes; the female glands are in the middle of the poral side of the segment; the uterus is a transverse tube extending beyond the osmoregulatory canal; and the ventral osmoregulatory vessel is situated laterally to the dorsal canal. *Parabertiella* differs from *Perutaenia* in that the sexual pores are irregularly alternate; the testes are placed in only one field; the female glands in the middle of the aporal side of the segment; and the uterus is a transverse tube which extends beyond the osmoregulatory canals on both sides of the segment.

It should be noted that *Perutaenia threlkeldi* is much smaller than the other cestodes which have been described as belonging to the subfamily ANOPLOCEPHALINAE.

SUMMARY

A new genus and species *Perutaenia threlkeldi,* of the subfamily ANOPLOCEPHALINAE, is described from *Lagidium peruanum* Meyen from the Sierra of Peru, South America.

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EXPLANATION OF PLATE

FIG. 1. Entire strobila of *Perutaenia threlkeldi.*

FIG. 2. *Scolex of P. threlkeldi.* Note evidence of segmentation immediately behind scolex.

FIG. 3. Dorsal view of mature proglottids of *P. threlkeldi* showing the testes, cirrus pouch, ovary, uterus, and the osmoregulatory system.

FIG. 4. The cirrus pouch and everted cirrus of *P. threlkeldi.* Note the internal and external seminal vesicle and the spines on the cirrus.

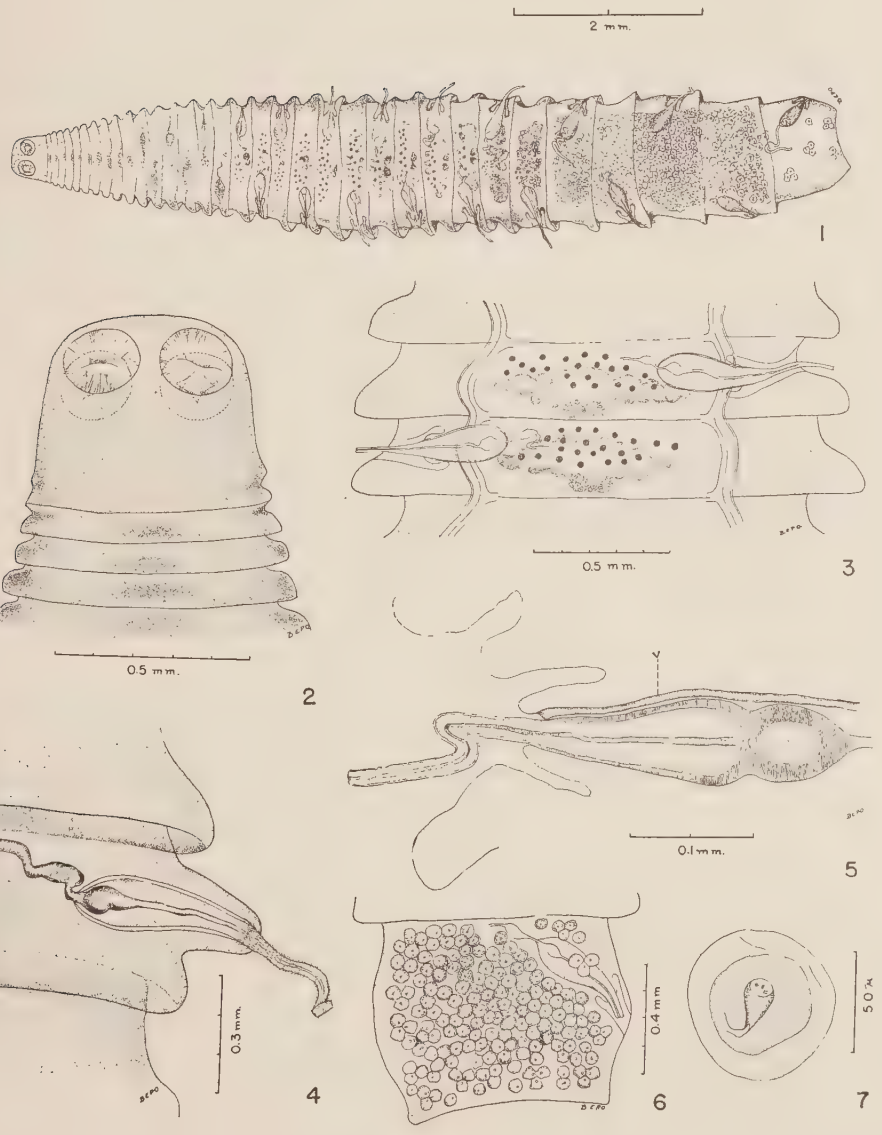
FIG. 5. Composite drawing of the cirrus pouch and vagina (v) of *P. threlkeldi.* Reconstructed from longitudinal sections.

FIG. 6. Gravid segment of *P. threlkeldi.*

FIG. 7. Egg of *P. threlkeldi.*

Figure 1 was drawn with the aid of a projector. Other figures were drawn with the aid of a camera lucida.

Plate I



ORNITHODOROS SALAHI SP. NOV. (IXODOIDEA, ARGASIDAE) FROM THE CAIRO CITADEL, WITH NOTES ON *O. PIRIFORMIS* WARBURTON, 1918 AND *O. BATUENSIS* HIRST, 1929

HARRY HOOGSTRAAL

U. S. Naval Medical Research Unit No. 3, Cairo, Egypt

A distinctive tick from the Citadel and nearby areas in Cairo, Egypt, is an undescribed member of an Asiatic group characterized by: small size; piriform shape; absence of eyes, cheeks, and distinct hood; small, inconspicuous discs or no apparent discs; absence of dorsocentral tarsal humps but with small subapical hump on tarsus I; and mammillated integument. The new Egyptian species is morphologically close to *O. batuensis* Hirst 1929 and to a very closely related, yet unnamed Philippine species. These three species have many characters in common with *O. piriformis* Warburton 1918, but lack the large, very distinct integumental discs of *O. piriformis*. Bats are known or believed to be the hosts of all these species.[1]

Ornithodoros salahi sp. nov.

Holotype: Engorged male, Cairo, Egypt, on wall in large hall under Mohammed Ali Mosque, Citadel area, 9 May 1951, by H. Hoogstraal. Deposited in the United States National Museum, No. 2008.

Allotype: Female, same data as holotype.

Paratypes: 400 males, 400 females, 400 nymphs, 80 larvae, collected on walls or floors in same place as holotype, or in nearby Sultan Hassan Mosque, and under *Fom el Khalig* aqueduct in Old Cairo, at various times in 1951 and 1952 by H. Hoogstraal, Abdel Aziz Salah Effendi, Sayed Mittwally, Ibrahim Soliman Khetr, and Sobhey Gaber. Also 300 laboratory-reared larvae, mounted on slides, from paratype parents. Specimens deposited in collections of United States National Museum; Fouad I Entomological Society, Cairo; Rocky Mountain Laboratory, Montana; Museum of Comparative Zoology, Harvard University; Chicago Natural History Museum; British Museum (Natural History); Dr. R. B. Heisch, Nairobi; Division of Veterinary Services, Onderstepoort; the writer, and other persons.

Description. Male: Average 3.4 mm. long, 2.0 mm. wide (range 3 to 3.9 mm. long, 1.6 to 2.6 mm. wide). *Body* (engorged) oval, broadly rounded posteriorly, narrowly rounded anteriorly, (unengorged) piriform; *color* in life normally purple to black, legs pale yellow. *Dorsal integument* with dense, narrowly separated, very slightly elevated, jaggedly circular mammillae, the center of each with a small, hard shiny black knob of variable shape; some lateral mammillae with a very small central hair, lateral mammillae scale-like, varying from broadly rounded to broadly conical, but none narrow or spine-like. (In living and freshly preserved specimens, mammillae appear as pale, slightly raised areas with irregular, jagged outlines, narrowly separated by darker integument). *Discs* small, inconspicuous, slightly depressed, minutely

Received for publication, August 11, 1952.

Research Project No. NM 005 050.29.11.

The opinions and statements contained herein are the private ones of the writer and are not to be construed as official or reflecting the view of the Navy Department or the Naval Service at large.

[1] Since this manuscript was submitted, larvae have been found in fruit bat roosts in the Aquarium grotto, Cairo, and in Wadi Natroum, Western Desert, Egypt. I am informed that Professor O. Theodor has compared Egyptian specimens with specimens collected in Jerusalem and has found them to be the same.

reticulated, black, without raised borders; more or less easily visible in living and freshly preserved specimens, almost invisible, or invisible in most preserved opaque specimens. *Ventral integument* with smaller, more distant mammillae grading into striated, unmammillated areas, a dense patch of very small hairs anterior of capitulum.

Capitulum small, lying in deep camerostome, entirely obscured from dorsal view except when tick is grossly engorged; *basis capituli* commencing at level of middle or basal half of trochanter I, surrounded by membranous collar at rest (this collar expanded to form swollen tube when feeding), cheeks or lateral sclerites absent. *Hypostome* 0.15 mm. long, slightly notched apically, denticles only on apical half, 2/2, lateral file of five denticles progressively larger from apical to basal, internal file of five weaker, smaller denticles (in some specimens four or five very tiny, weak median pairs are also present); corona with five rows of five or six hooklets on each half. *Palpi* about 1.50 as long as hypostome (Figure 5 shows palp fore-shortened in normal curved position); width of each segment slightly more than half length; basal segment largest, apical segments each about 0.75 size of preceding one. *Postpalpal hairs* five, the longest reaching apical margin of palpal segment II; *posthypostomal hairs* two, as long as hypostome.

Eyes absent. *Spiracular plates* laterad of coxa IV. *Genital aperture* with straight posterior border, broadly rounded anteriorly, situated at basal level of coxae I in a keyhole-shaped area of rugose integument. *Anus* elliptical, a long, fine, posterior and anterior hair on each valve. *Preanal grooves* interrupted medially, slightly curved, deeply cleft, situated just posterior of anus. *Median postanal groove* with seven or eight irregular pairs of very small discs. (These discs are usually discernible only following dropping in alcohol and for one or two days afterwards before the integument becomes opaque.) *Transverse postanal groove* at distal tenth of body, rugose anterior lip divided medially by median postanal groove, posterior lip rugose medially. *Dorsoventral groove* absent. *Coxal fold* extending from level of coxae I to transverse postanal groove, with five irregular rows of short, yellowish hairs. *Supracoxal fold* present.

Legs fairly long; leg IV almost 0.75 length of body, other legs about 0.75 as long as IV; surface with rows of short hairs becoming longer on apical segments. *Coxae* subequal, I and II separated; II, III, and IV contiguous. *Tarsi* II, III, and IV gradually taper on distal two-fifths to narrow tip, dorsal armature absent. *Tarsus I* with dorsal and ventral surfaces parallel on basal half; distal dorsal surface (and sometimes ventral surface) slightly expanded to Haller's organ; subapical hump small, truncate, projecting dorsally only as much as area immediately anterior of Haller's organ; distal vertical margin of hump straight or slightly concave, two-fifths as long as width of segment; apical fifth of tarsus abruptly tapered. In many large specimens (of both sexes) the subapical hump is slightly more elevated and the gap marking the opening of Haller's organ more definite; this, with the slight concavity of the distal vertical margin of the subapical hump, causes the hump to appear more pronounced than is illustrated for a tick of average size (Figure 1), and to appear slightly tilted. *Claws* one-fourth length of tarsi, sharply curved apically.

Female: Average 4.6 mm. long, 2.6 mm. wide (range 3.8 mm. to 5.2 mm. long, 2.1 mm. to 3.3 mm. wide). Anterolateral (or peripheral) area with rounded, scale-like mammillae interspersed with a few more acutely pointed mammillae. Otherwise similar to male except for generally larger size, often wider in outline. Genital aperture a long narrow slit level with apex of trochanter II in most specimens, in some it is very slightly anterior, rarely slightly posterior of this level.

Nymph: Similar to adults except for size and absence of genital aperture; hypostome proportionately larger than that of adult, apex of palps often extending beyond anterior margin of body.

First stage nymphs measure from 1.7 to 1.9 mm. long, 0.8 to 1.1 mm. wide.

Larva: (Unengorged). Length (excluding mouth parts) 0.45 mm., width 0.25 mm. Body with parallel sides, slightly converging anteriorly to basis capituli, bluntly rounded posteriorly. Integument very faintly striated; three pairs of long posterior hairs; dorsally with nine pairs of almost equidistantly spaced sublateral hairs and one median pair at about level of posterior sublateral pair; ventrally with seven pairs of hairs just mesad of coxae and a single median pair at basal level of coxa I; faint suggestion of coxal fold visible. Legs robust, almost as long as body including capitulum; tarsal shape suggestive of that in subsequent stages; coxae elongately triangular, equidistantly spaced, each with three subapical hairs. Anus midway between coxa III and posterior margin of body, elliptical, with a single pair of long hairs. *Capitulum.* Basis capituli almost as wide as body, basal margin straight dorsally, slightly convex ventrally; lateral margins almost parallel, anterior margin forming an obtuse angle. Hypostome measures 0.12 mm. long, 0.05 mm. wide; robust, with strong denticles from base to corona; apex without notch, varying from rather acutely to (usually) bluntly rounded; corona apically without hooklets or with a few minute ones in irregular rows which are strongest laterally, basally with two rows of

eight or ten hooklets and four or six others scattered basally; denticles 2/2, eight pairs of large, deeply separated, lateral denticles grading in size from small apically to large basally, four or five pairs or smaller interior denticles. *Palpi* about as long as hypostome, with three subequal, robust basal segments, apical segment narrower.

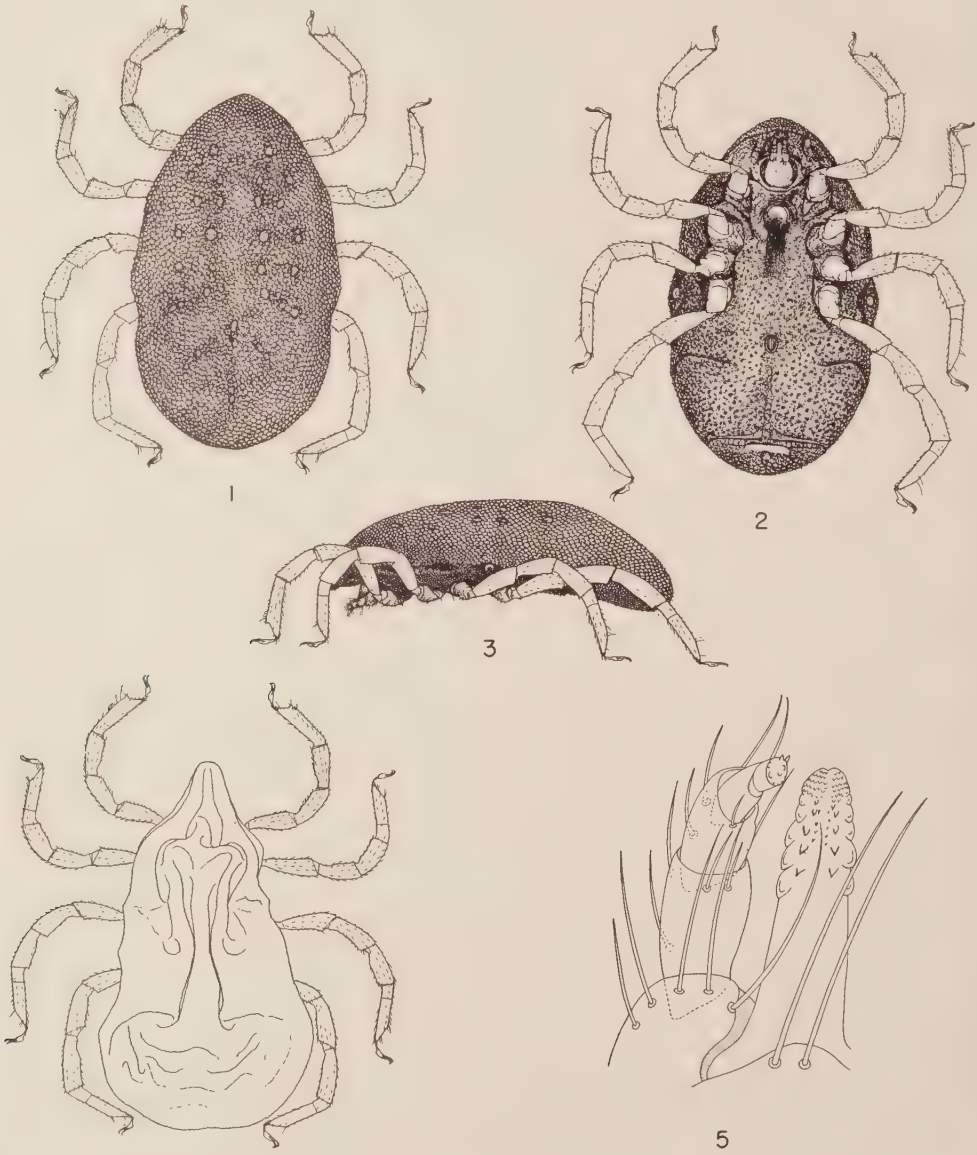


FIG. 1. Male, engorged, dorsal. FIG. 2. Male, engorged, ventral. FIG. 3. Male, engorged, lateral. FIG. 4. Male, emaciated, dead, dry (diagrammatic). FIG. 5. Male, hypostome and palp, ventral.

Larva: (*Engorged state removed from host.*) Measures (exclusive of mouth parts) 0.90 mm. to 1.35 mm. long, 0.50 to 0.70 mm. wide; color shiny brownish-red to amber, legs pale yellow. *Body* elongate-oval, bluntly rounded posteriorly, more narrowly rounded anteriorly (greatly engorged specimens often with truncate anterior margin); integument finely striated, deeply furrowed as illustrated. *Body hairs:* three long posterior pairs, three lateral pairs near level of

legs II and III, two lateral pairs near level of leg I, and two pairs near base of palpi; ventrally a median pair just posterior of basis capituli, seven median pairs from base of coxa I to posterior border (other hairs missing from almost every specimen examined though the follicle is often visible). Lateral surface of body meets dorsum at right angles, curves ventrally to venter. *Capitulum* arises from anterior surface of anteriorly truncate specimens, from latero-ventral surface of anteriorly rounded specimens; basis capituli a subrectangular shield visible only ventrally. Hypostome and palpi as in unengorged state. *Anus* slightly posterior of level of leg III; deep median furrow extending from anus nearly to posterior body margin; lateral paired furrows slightly posterior of anus. *Legs* about 4/5 as long as greatest width of body; equidistantly spaced on anterior half of ventrum.

Host: Egyptian fruit bat, *Rousettus egyptiacus* (E. Geoffroy). Other bats apparently only when they rest near fruit bats.

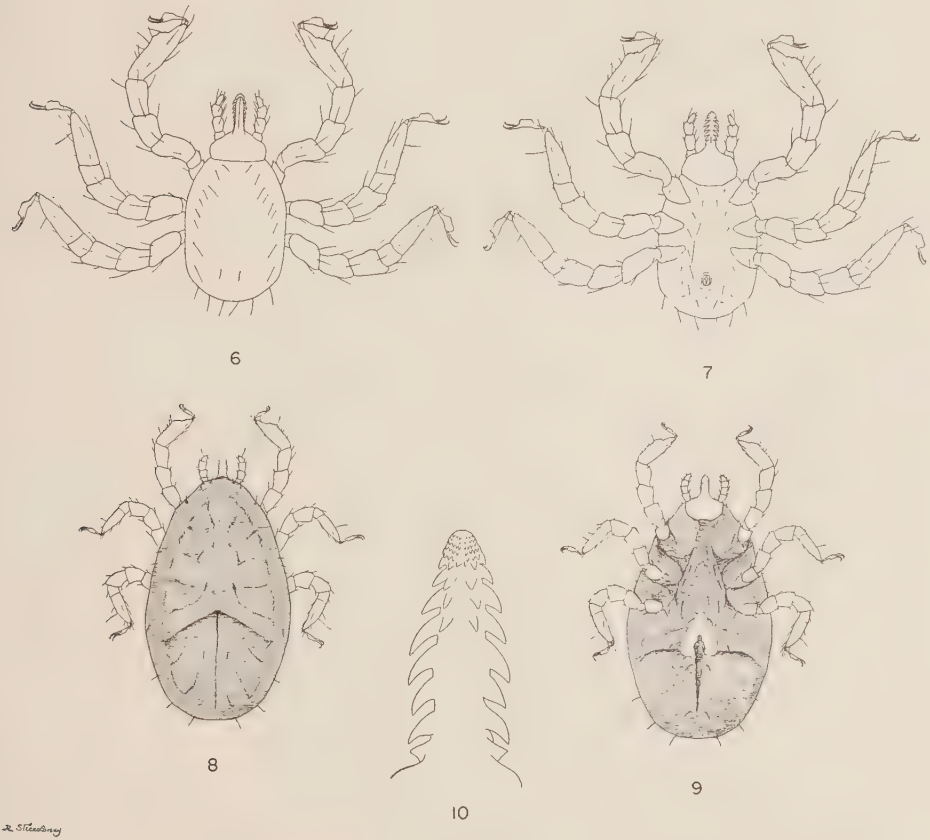


FIG. 6. Larva, unengorged, dorsal. FIG. 7. Larva, unengorged, ventral. FIG. 8. Larva, engorged, dorsal. FIG. 9. Larva, engorged, ventral. FIG. 10. Larva, hypostome.

Related species: This species has many characters in common with *O. piriformis* Warburton 1918, known only from the type collection from Mahabaleshwar, Satara District, India. Examination of the type female and two cotype females of *O. piriformis* in British Museum (Natural History) shows that this species differs from *O. salahi* in that the discs are very distinct and at least twice as large, and that they are rimmed by a raised border (like a miniature lily pad). Mammillae of *O. piriformis* are much larger, sparser, and more widely separated. Furrows between individual mammillae are not jagged and are deeper than those of *O. salahi,* but not the deep intermammillary grooves of *O. batuensis* (discussed below). Peripheral

mammillae of *O. piriformis* are elongately triangular, or spine-like, and densely grouped. Though the legs are more robust, they are only about three-fourths the length of those of *O. salahi.* Hairs on the ventral surface and legs are at least twice as long as those of *O. salahi.* The absence of remarks about these characters in the original description would lead one to believe that *O. piriformis* is much closely related to *O. salahi,* and to *O. batuensis,* than it actually is.

Warburton's (1918) illustration differs in certain details from characters in the type series of *O. piriformis.* In these specimens, the spiracles are at the same level as in *O. salahi,* i.e., level with the posterior leg, not far distad of it as illustrated. The median postanal groove contains discs and divides the anterior lip of the transverse postanal groove, as it does in *O. salahi.* The dorsal subapical protuberance of hairs; leg IV of female 1.10 as long as (0.75 in *O. salahi*); male mammillae more tarsus I is not a narrow, knob-like hump as in the original illustration, but is actually like that of *O. salahi.*

O. salahi is related to *O. batuensis* Hirst 1929, known only from the type locality, Batu Caves (Dark Cave), Selangor, Federated Malay States. The material identified as *O. batuensis* from Davao Province, Mindanao and Samal Islands, Philippine Republic (Kohls 1950) is actually a closely related, undescribed species which Mr. Kohls will subsequently describe. The *O. batuensis* material in British Museum (Natural History) consists of the type female and a cotype nymph, besides a male collected later in the same caves. Although the male specimen was collected and sent to the Museum before the description was published, it was not mentioned in the original description. It is marked as a cotype, but can hardly be considered as more than a topotypic specimen.

Examination of the above *O. batuensis* material showed the following characters differing from *O. salahi:* both sexes with discs of the *O. salahi* type (as opposed to the *O. piriformis* type) but these discs much more distinct than in *salahi,* in which they almost entirely disappear from sight after preservation; longer body and leg conical and elevated on posterior half of dorsum; intermammillary grooves of both sexes very deep and almost as wide as individual mammillae (in *O. salahi* these are merely very shallow, narrow furrows); females with numerous spine-like peripheral mammillae, males with numerous narrow, compressed, elongate peripheral mammillae (these absent in *O. salahi*).

The undescribed Philippine species is much more closely related to *O. batuensis* than it is to *O. salahi.*

Biology. Although we have searched in scores of caves and niches in the Cairo area and in an equal number in other parts of Lower Egypt over a two year period, *O. salahi* has been found only in three places in the city itself. It is fairly common in the basement of Mohammed Ali Mosque in the Citadel area where large swarms of fruits bats, *R. egyptiacus* (E. Geoffroy), rest at the top of huge, high-ceilinged, cliffside rooms supporting the edifice. Fewer are found in chambers where these bats roost in nearby Sultan Hassan Mosque. The largest local population is under the supports of *Fom el Khalig* aqueduct on the riverfront in Old Cairo, where the same bat species rests.

About a hundred larvae in various degrees of engorgement have been taken from two hundred fruit bats that have been examined.

Only one larva of this spceies has been seen on almost 5000 other bats, represent-

ing a dozen species, that we have examined in the Cairo area. The single exception was found on a tomb bat, *Taphozous perforatus perforatus* E. Geoffroy, from Sultan Hassan Mosque. This host was in a group resting near fruit bats. Because other bats very seldom rest near fruit bats in this area, and we have found larvae on none other than fruit bats and the single neighboring tomb-bat, it seems reasonable to assume that fruit bats are the chief hosts and other kinds are attacked only under special local conditions.

Engorged larvae can easily be found among moist bat droppings on the floor at each site where fruit bats rest.

Nymphs and adults rest among bat droppings, under rocks, or in lower wall crevices. They commence crawling upwards on walls toward midday. This movement is so definite that when we were collecting only at Mohammed Ali Mosque, where careful search is necessary to find any numbers of ticks, we scheduled trips to late morning in order to save time. Later, when we collected hundreds of specimens from *Fom el Khalig* aqueduct and confined them in tubes, some tendency to a similar increase in late morning climbing activity was observed. Hungry ticks apparently seek bats near the roof at the time of day when hosts are resting most quietly, but retreat to or near ground level after feeding.

The winter population in *Fom el Khalig* aqueduct consisted of 3.5 times as many nymphs as adults.

A single nymph has been found crawling on a street in Heliopolis, a suburb of Cairo. It had probably fallen from a flying bat.

In Cairo city proper, this is by far the most common tick parasite of bats. Specimens of *Argas boueti* Roubaud and Colas-Belcour, 1933 and of *Argas vespertilionis* (Latreille, 1796) (or *testudo* Rossi, 1790) are sometimes found with *O. salahi,* but the third reported bat tick of this area, *Argas transgariepinus* White, 1846, has not been found in association with it (Hoogstraal 1952).

Mating position is as common for the genus. Mating activity of pairs confined in tubes often continues for as long as eight hours, and certain pairs frequently join over a period of at least two weeks.

Individual eggs are round or slightly ovoid and measure about 0.22 mm. in diameter. Some females begin to lay eggs during the middle of April but most begin during the first week of May; after June fewer eggs are laid, and none after September (1951–2 observations). Egg batches, laid at night, consist of from 22 to 54 eggs. Larvae emerge after 11 to 16 days. More detailed life cycle studies are now in progress.

After laying eggs, females "brood" over them until the larvae hatch (Figure 11). Some females return to the egg batch when disturbed but others abandon them. This phenomenon is now known for a large number of argasid species.

Feeding. Nymphs and adults feed readily on human beings or bats but only erratically on baby white mice or rats. During feeding, ticks usually lunge forward at intervals of about four seconds. Repletion is reached in half an hour, but bleeding from the wound, which commences when the mouthparts are released from human beings, may last for another half hour. Little or no bleeding has been observed after feeding on bats. Coxal fluid has not been observed during or after feeding, and none has been demonstrated by several usual techniques to excite the flow of this sub-

stance in *Ornithodoros* ticks. What appears to be a minute coxal gland aperture is visible in living specimens just posterior of the base of coxa I.

Slight irritation is felt at the site when mouthparts are inserted. Itching in the area surrounding the wound continues for as long as five weeks afterwards. The site is marked by a pink papule with a small reddish center. Some wounds disappear in a few weeks, others remain fairly prominent for as long as six months.

Almost every time that members of our staff have returned from a collecting trip for this species, nymphal or adult specimens of both sexes have been found feeding on the arm, hand, neck, axilla, thigh, groin, or abdomen. One assistant attributed

FIG. 11. Female "brooding" eggs laid the previous day. (Official U. S. Navy Photograph by HMC Cloyce P. A. Strome).

enlarged inguinal glands to two bites on the thigh and groin, but we have no proof that these bites caused the condition. Workmen who are at present repairing *Fom el Khalig* aqueduct tell us that they very frequently find these ticks on themselves and that many engorge.

A histopathological study (by Dr. Paul Hamilton of U. S. Naval Medical Research Unit No. 3) of several bites of adult *O. salahi* on wing membrane of a tomb bat, *Taphozous p. perforatus,* shows marked hemorrhage between epidermis and skeletal muscle. There were a few focal aggregates of polymorphonuclear leucocytes and marked edema in the skeletal muscle surrounding the hemorrhage. Within the hemorrhage, there were several small, yellow fragments of refractile

foreign bodies which are presumed to be portions of the ticks (although the ticks disengaged voluntarily after feeding).

Thirty emulsified adult specimens have been examined under dark field illumination but no spirochetes have been discovered.

Acknowledgments. This species is dedicated to my colleague Abdel Aziz Salah Effendi, Chief Technical Supervisor of Field Investigations on loan from the Egyptian Ministry of Health to U. S. Naval Medical Research Unit No. 3. The faithful, efficient, and self-effacing service he has rendered his Government and this Unit during the several years he has been assigned in this position has greatly furthered medical research in Egypt.

Recognition is due Dr. Abdel Naby El Nahas, In Charge, Royal Memorial Palaces, for permission to investigate the chambers under Mohammed Ali Mosque; to Mr. E. Browning, British Museum (Natural History), for making the initial comparisons with the related species and later for facilities for me to examine the *O. batuensis* and *O. piriformis* type series at the Museum, and to Mr. Glen M. Kohls, Rocky Mountain Laboratory, Hamilton, Montana, for critical comments and loan of Philippine *O. batuensis* specimens.

Remarks. Interested specialists will be furnished specimens of this species upon request.

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NOTES ON THE TICKS OF GUAM WITH THE DESCRIPTION OF *AMBLYOMMA SQUAMOSUM* N. SP. (ACARINA: IXODIDAE)*

GLEN M. KOHLS

Four species of ticks, *Boophilus annulatus* (Say), *B. microplus* Canestrini, *Rhipicephalus sanguineus* (Latr.) and *Amblyomma cyprium* Neumann, have been previously reported as occuring on Guam. References to the occurrence of the cattle ticks, *B. annulatus* and *B. microplus* (= *Margaropus annulatus australis,* synonym) are found in the Reports of the Guam Agricultural Experiment Station (Fullaway, 1912; Thompson, 1913; Edwards, 1919). According to Barber (1916), the cattle ticks were considered by Dr. B. H. Ransom to be indistinguishable from *Margaropus caudatus,* a species which the present writer regards as identical with *B. microplus.* Horses, carabaos, goats, and deer were also said to be infested with this tick. Vandenberg (1929) reported the presence of the brown dog tick, *R. sanguineus,* on dogs, and Alicata (1948) reported that "examination of one bull near Agaña, Guam, revealed a moderate infestation of ticks identified as *Boophilus annulatus australis* (Fuller) and *Amblyomma cyprium* Neumann." Dr. Alicata states (personal communication, 1952) that his report of *A. cyprium* was based on a single male specimen. The statement in War Department Technical Bulletin, TB Med. 57 (1944) that "Cattle in Guam frequently are infested with ticks of the family Argasidae" is undoubtedly in error and in all probability it refers to ticks of the genus *Boophilus.*

Collections made on Guam by personnel of Naval Medical Research Unit No. 2 in 1945 were presented to the Rocky Mountain Laboratory by the late Dr. A. B. Hardcastle, a former member of the Unit. These contained two additional species, *Ornithodoros capensis* Neumann and a new species of *Amblyomma.* The identity of the former is based on a single adult reared from larvae off the noddy tern, *Anous stolidus,* Ypao Point and Oca Point, May, June, and July. The latter, described below, was found on the water-monitor, *Varanus indicus,* at Tarague Point and Pati Point in June and July. Also represented were immature stages which appear to be those of the previously recorded *A. cyprium,* the larvae and nymphs of which are undescribed, off *Rattus rattus* and "rats," Ritidian Point and an unnamed locality, June and September.

Amblyomma squamosum n. sp.

Female

(Fig. 1, A–F)

A small tick; length excluding capitulum and width of the two specimens available, both partially engorged, 3.3[1] by 2.2 (holotype) and 3.7 by 2.6 (paratype). Dorsum exclusive of scutum densely clothed with conspicuous pale ovate scales. Venter posterior to the spiracles similarly clothed and with a band of smaller hairs extending forward on each side on the area between the coxae and the genital groove to about the level of the genital aperture. Dorsal foveae unusually large and distinct as two salient plaques posterior to the scutum.

Capitulum: Greatest width of basis 0.68. Basis subrectangular, broader than long, posterior

Received for publication, August 25, 1952.

* From the Federal Security Agency, Public Health Service, National Institutes of Health, National Microbiological Institute, Rocky Mountain Laboratory, Hamilton, Montana.

[1] All measurements in millimeters.

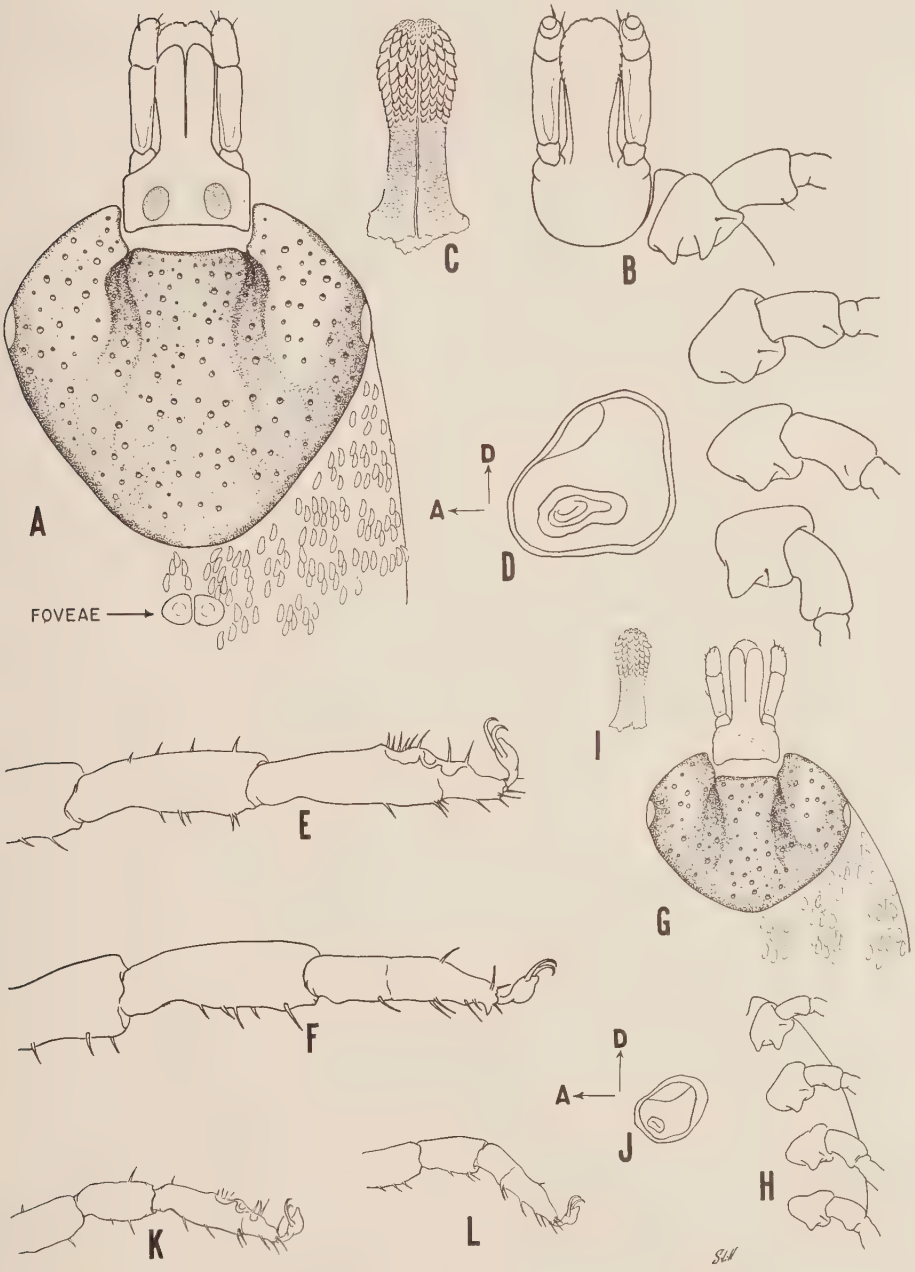

FIG. 1. *Amblyomma squamosum* n. sp. A. Female capitulum, scutum, and portion of body showing scales and the foveae. B. Female capitulum and coxae, venter. C. Female hypostome. D. Female spiracular plate (a = anterior, d = dorsal). E. Female metatarsus and tarsus, leg I. F. Female metatarsus and tarsus, leg IV. G. Nymph capitulum, scutum, and portion of body showing scales. H. Nymph coxae. I. Nymph hypostome. J. Nymph spiracular plate. K. Nymph metatarsus and tarsus, leg I. L. Nymph metatarsus and tarsus, leg IV.

margin between the slightly salient posterolateral angles nearly straight; punctations absent on holotype but a few are present between the porose areas of the paratype. Porose areas small, depressed, subcircular, and separated by a distance of a little more than the diameter of one. Palpi slender, 0.76 in length, article 2 about twice as long as article 3. Hypostome mildly notched, the principal denticles arranged 4/4 and limited to the terminal half.

Scutum: Subtriangular, 1.67 long by 1.77 wide (holotype) and 1.84 long by 1.90 wide (paratype). Posterior angle broad with the apex broadly rounded, posterolateral margins nearly straight. Cervical grooves deep anteriorly and continuing posteriorly as broad shallow depressions to near the margin of the scutum. Punctations numerous, unequal in size, and rather evenly distributed. Scutum ornate, with an irregular pale spot in the anterolateral fields and a much smaller spot in the posterior angle. Eyes broad, flat, and pale.

Legs: Coxae I with two moderate subequal, blunt spurs; II, III, and IV each with a spur similar to those of coxae I. Apical and subapical ventral spurs absent on tarsus I; apical ventral spur present on II, III, and IV. Length of tarsus I 0.87; metatarsus 0.66. Length of tarsus IV 0.64; metatarsus 0.66.

Genital aperture: Located between coxae II.

Spiracular plate: Shape as figured. Greatest length 0.65, greatest width 0.50.

Male unknown.

Nymph

(Fig. 1, G–L)

The four available specimens are partially engorged, the one least engorged is 1.40 long, excluding the capitulum, by 1.14 wide. The body is clothed with scales as in the female except that the scales are absent on the venter anterior to the spiracles.

Capitulum: Length 0.56, width of basis 0.34. Basis subrectangular, mildly convex dorsally and with a few scattered punctations. Posterior margins between the mildly salient posterolateral angles nearly straight. Palpi long and slender, combined length of articles 2 and 3 about 0.40. Hypostome with the principal denticles arranged 3/3.

Scutum: Length 0.75, width 0.95. Shape much as in the female. Ornate, with an irregular pale spot in the anterolateral fields. Cervical grooves deep anteriorly, extending as broad shallow depressions to near the posterior margin. Punctations similar to those in the female. Eyes large and a little convex.

Legs: Coxal spurs as in the female. Apical and subapical ventral spurs absent on all tarsi. Length of tarsus I 0.45; metatarsus 0.25. Length of tarsus IV 0.35; metatarsus 0.24.

Spiracular plate: Shape as figured. Greatest length 0.26, greatest width 0.20.

Holotype: Female, from the water-monitor, *Varanus indicus,* Pati Point, Guam, June 4, 1945. Deposited in the Rocky Mountain Laboratory, Hamilton, Montana (No. 27509).

Paratypes: One female and 3 nymphs with data as for the holotype. One nymph, host as above, Tarague Point, Guam, July 12, 1945. All in the collection of the Rocky Mountain Laboratory (No. 27526).

The unusually large and distinct dorsal foveae of the female suggest a relationship of the new species to *Amblyomma malayanum* Neumann 1908 (= *caelaturum* Cooper and Robinson 1908, synonym), to which it keys in Robinson (1926). It is readily distinguished from this species, however, by the peculiar scales on the body and by its very much smaller size. Furthermore, the hosts of *A. malayanum* so far as known are tortoises and the species is known to date only from the Federated Malay States, Singapore, Sumatra, Philippine Islands (Anastos, 1950), and Borneo (Schulze, 1936) and Kohls (unpublished data). The nymph of the new species is one of the very few ornate nymphs that are known in this genus and like the female it too is distinctive in having the body clothed with scales.

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A SEARCH FOR DRUG-FAST STRAINS OF *EIMERIA TENELLA*

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Coccidiostatic drugs have come into widespread use because most epidemics of avian coccidiosis last for several weeks. Therefore, therapeutic treatments of a few days duration cannot prevent the major portion of the losses. But continuous medication with a coccidiostat suggests the possibility that these protozoa may become drug-fast. Furthermore, numerous complaints from poultrymen suggested to us that at least one drug might be less effective than formerly. Therefore, tests were designed to explore the possible existence of drug-fastness.

MATERIALS AND METHODS

The chicks employed were obtained as day-old birds from a local hatchery and kept in a wire-floored, battery brooder. They were banded and weighed individually when from 10 days to 3 weeks of age. They were divided into 10 groups according to weight, and each group was confined in a separate cage.

The coccidia were of four strains having the following histories: The Standard Strain was isolated in our laboratory more than ten years ago. It has been reproduced without contact with any coccidiostatic drug throughout that period.

The Nitrofurazone Strain was isolated in January of 1952 from one of the brooder houses operated in connection with this laboratory. There are 3 of these brooder houses which have been used to explore the coccidiostatic properties of nitrofurazone for three years. This coccidiostat has not been used for nearly so long a time at any other site.

The Lunceford Mixed Strain was isolated from poultry litter received from Georgia. Because poultrymen have frequently complained that sulfaquinoxaline is not as effective as when first used, about four years ago, we have searched for a strain of *Eimeria tenella* which has been exposed to this drug for several years. Mr. William Lunceford collected, at our request, a sample of litter from a broiler plant in Georgia where sulfaquinoxaline has been used regularly as a coccidiostat since the drug was first sold to the public. This litter was fed to coccidia-free chickens, and the mixed culture of *Eimeria tenella* and *Eimeria necatrix* obtained is designated as the Lunceford Mixed Strain.

The Lunceford Strain is a pure culture of *E. tenella* which was isolated from this mixed culture.

The numbers of sporulated oocysts were determined for each culture employed. Four days after weighing, banding and sorting into 10 groups, each bird was given equivalent doses of the cultures under test. The birds in 5 of the cages were given the Standard Strain and those in the other 5 cages were given one of the newly-isolated cultures. On the day of infection, the drugs were freshly mixed with the feed and given to the birds shortly after the infective doses were administered. Medication was continued for 6 days.

Received for publication, October 6, 1952.

Nitrofurazone and sulfaquinoxaline were used in each test. Both drugs were employed at the prophylactic levels recommended by the manufacturers. Since the manufacturers of sulfaquinoxaline changed their recommendations while these tests were in progress, that drug was used at two levels, namely, 0.0125 per cent and 0.015 per cent of an all-mash feed. Since the birds were given massive doses of sporulated oocysts, we anticipated that the controls would suffer a mortality of 50 per cent or more; that the prophylactic drugs would reduce this mortality by one-half or two-thirds. If a drug-fast strain of coccidia were encountered, we anticipated that the reduced efficacy of the drug would be manifested by increased mortality in medicated cages infected with the fast or resistant strain. Therefore, our method parallels closely the methods used by several entomologists to demonstrate the presence of DDT-resistant houseflies in the field.

The chicks were weighed at frequent intervals and these objective criteria were also employed to estimate the significance of the data. All birds which died were necropsied and the cause of death ascertained to be coccidiosis by the lesions present in the intestinal tract.

EXPERIMENTAL RESULTS

The data obtained from the three experiments are summarized in table 1. A limited description of each experiment is necessary to complete the tabular presentation.

Experiment 1. In this test 100 chicks received each culture. Sixty-two per cent of the 100 chicks receiving either the Standard culture or the Newly isolated cultures died of the disease. The results indicate that the two cultures were of equal virulence. Furthermore, deaths within the cages receiving medication were equally distributed between the cages receiving the Standard culture and those receiving the New culture. Therefore, there is no suggestion of drug-fastness.

These tests also give some evidence of the efficacy of the drugs when used at prophylactic levels. Of the 80 controls, 71 chicks, or 88.75 per cent, died of coccidiosis. Of 80 chicks given nitrofurazone in the mash, only 25, or 31.25 per cent, died. The difference is highly significant. In this test sulfaquinoxaline was used in two cages as a reference control at the level formerly recommended by the manufacturers for prophylaxis. Of the 40 reference controls, 28 died of the disease. By the chi-square test this death rate differs significantly from that of the controls and of the nitrofurazone-treated birds.

Experiment 2. The Lunceford Mixed culture was tested comparatively with our Standard culture in this test, although the two cultures are not strictly comparable. At necropsy of the birds it was noted that lesions commonly associated with the species *E. necatrix* were present. Therefore, the Lunceford Mixed culture contained a large percentage of *E. necatrix* oocysts, when used in this test. Thirty-nine per cent of the birds receiving the Standard culture died of the experimental infection, but only 16 per cent of the 100 chicks receiving the Lunceford Mixed culture died. The difference is highly significant by the chi-square test. Presumably this difference is due to the admixture of *E. necatrix* in the Lunceford Mixed culture. However, the reduced number of deaths among the birds receiving the Lunceford Mixed culture was not due to the resistance of the chicks receiving medication. Thus among the control chicks, 21 out of 40 chicks receiving the

Standard Culture died, but only 8 out of 40 died among those receiving the Lunceford Mixed culture. The ratio is as 2.6 : 1. In each of the medicated groups the similar ratio is as 2.25 : 1, or slightly lower. Therefore, no evidence of drug-fastness can be obtained from this test.

Again the experiment yields evidence of the comparative efficacy of the two drugs. Twenty-nine chicks out of 80, or 36.25 per cent, died of the experimental infection in the four control cages; 13 out of 80, or 16.3 per cent, died in the four nitrofurazone cages. The difference is probably significant since chi-square (using the Yates correction) equals 6.23. Thirteen chicks, or 30.25 per cent, of 40 reference controls died (receiving 0.015 per cent sulfaquinoxaline). The death rate among these did not differ significantly from either the unmedicated controls or from the nitrofurazone medicated birds.

Experiment 3. This test is a comparison of the Standard Strain with the Lunceford Strain of *E. tenella.* Fifty-seven out of 95 birds died among those receiving the Lunceford culture, and 47 died out of 95 birds receiving the Standard culture. This difference is not significant by the chi-square test and presumably is due to chance factors. Since the Lunceford Strain proved the more virulent among all three treatments there is again no evidence of drug-fastness. The ratios of death (Standard Strain/Lunceford Strain) within each treatment are as follows: Controls 1 : 1.25; nitrofurazone medicated chicks 1 : 1.25 and sulfaquinoxaline medicated birds 1 : 1.16.

The comparative efficacy of the two drugs follows closely the pattern established in the preceding experiments. Of 76 birds in each group, 54 of the controls and 41 of those receiving 0.015 per cent sulfaquinoxaline died. The difference in deaths is probably significant since chi-square equals 4.46. Of 38 birds given 0.0055 per cent of nitrofurazone in the feed, 9 died of coccidiosis. This number of deaths is probably significantly fewer than the number of deaths in the cages treated with sulfaquinoxaline since chi-square equals 4.64. Obviously the frequency of deaths among the controls is significantly higher.

Since the data from all three tests point to similar conclusions, they may be combined in one table for ease of inspection. This has been done in table 1.

TABLE 1.—*Comparative efficacy of two drugs against strains of poultry Eimeria with and without prolonged exposure to coccidiostatic drugs*

Drug	Amount in feed (Per cent)	Number of birds infected with the		Number of deaths among birds infected with the		Combined Mortality (Per cent)
		Standard Culture	Newly isolated Cultures	Standard Culture	Newly isolated Cultures	
Nitrofurazone	0.0055	99	99	27	20	23.8
Sulfaquinoxaline	0.0125 to 0.015	78	78	41	41	52.6
Controls	.....	118	118	72	74	61.9
Combined mortality (per cent)						
Treated birds	.....			38.4	34.5	
Untreated birds	.....			61.0	62.7	

DISCUSSION

A small attempt was made to detect drug-fastness in *Eimeria* presumably exposed to coccidiostatic drugs. Drug-fastness would be apparent in our tests if mortality were excessive among the treated birds inoculated with the newly isolated cultures which had been exposed to prolonged drug treatment. However, mortality

in this group was actually slightly lower than mortality among the treated birds which received the standard culture. Therefore, no drug-fastness was detected by the methods employed. We suggest that the avian species of *Eimeria* have not yet acquired resistance to coccidiostatic drugs, but of course we cannot predict when drug-fast strains may appear in the field.

This study was stimulated by repeated complaints that certain coccidiostats are not as effective as formerly. Although some of the complaints of failure may have been justified, we suggest that the reasons for unsatisfactory performance bear no relation to drug-fastness. Indeed, other factors which may serve to discredit the continuous use of coccidiostats are easily identified. These include the use of inefficient drugs, use of efficient drugs at ineffective levels, inadequate mixing of effective drugs, and erroneous diagnosis of diseases which resemble coccidiosis.

Only two drugs, nitrofurazone and sulfaquinoxaline were tested. Under the conditions of our experiments, nitrofurazone proved much more effective than sulfaquinoxaline. Two hundred thirty-six birds were used as controls, and 146, or 61.9 per cent died of coccidiosis. One hundred fifty-six birds were treated with sulfaquinoxaline prophylactically. Of these 82, or 52.6 per cent, died. One hundred ninety-eight birds were given nitrofurazone prophylactically. Of these 47, or 23.8 per cent, died of the experimental infection. Therefore, the prophylactic levels of sulfaquinoxaline reduced the expected death losses by 9.3 per cent, and of nitrofurazone by 38.1 per cent.

SUMMARY

Nearly 600 chickens were used to ascertain if three newly isolated strains of *Eimeria* have acquired resistance to nitrofurazone or to sulfaquinoxaline. The experiments indicate that these strains were fully susceptible to the drugs named. Under the conditions of these experiments, the prophylactic levels of nitrofurazone proved much more effective for preventing deaths from experimental coccidiosis than the prophylactic levels of sulfaquinoxaline.

RAILLIETINA DEMERARIENSIS (CESTODA), FROM *PROECHIMYS CAYENNENSIS TRINITATUS* OF VENEZUELA

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INTRODUCTION

Cestodes collected by the Plague Mission to Venezuela 1950, which was sponsored by the Pan American Sanitary Bureau, the Venezuelan Government and the Bureau of Medicine and Surgery, U. S. Navy, were submitted to the writer by Doctor Ernst Schwarz, U. S. Naval Medical School, Bethesda, Md., for study and identification. The worms were found in the small intestine of a hystricomorph rodent, *Proechimys cayennensis trinitatis* (Or. No. 158; male; from Campamento Raphael Rangel, Sierra Maestra, Estado Aragua, Venezuela; altitude 1260 meters; collected by E. Schwarz, J. M. Amberson, H. K. Schwarz, July 27, 1950). Members of the genus *Proechimys* are found in tropical forest in Central and South America from Nicaragua to southern Brazil. Grateful acknowledgment is made to Doctor Schwarz for the opportunity to study this material. The tapeworms are referable to the genus *Raillietina* and to the subgenus *Raillietina* in which the genital pores are unilateral and each egg-capsule contains more than one egg. Specific determination, however, presents a very difficult problem.

The genus *Raillietina* is cosmopolitan in distribution; the worms infect birds and mammals and have been reported repeatedly from man. In a review of the genus, Hughes and Schultz (1942) listed 226 described species and many others have since been added. In human hosts the worms have been found only sporadically, in widely separated localities, and usually in small numbers. Joyeux and Baer (1929) postulated that rare cestodes of man are accidental infections by species naturally parasitic in other animals, especially rodents, which live in the same areas.

The first report of human infection by a species of *Raillietina* was given by Davaine (1870)* who described *Taenia madagascariensis* from two specimens passed by children living on the Comores islands near Madagascar. Blanchard (1891) transferred the species to the genus *Davainea,* and Fuhrmann (1920) included it in the new genus *Raillietina.* This or other closely related species have been reported from man and rats at various places in southern and eastern Asia. Leuckart (1891) reported it from man in Siam and Garrison (1911) from man in the Philippine Islands. The earlier accounts pertained to human infections but, as wild animals were examined for parasites, many species of *Raillietina* were described from birds and mammals of the Eastern Hemisphere (see Meggitt and Subramanian, 1927). López-Neyra (1930, 1931) suggested that many of the species described from Africa and Asia represent varieties of a single species which infects rodents and occasionally man. Narihara (1935) reported *R. madagascariensis* from both men and rats in Formosa, and Miyazaki (1950) found the species in *Rattus norvegicus* and *R. rattus* in Japan. Joyeux and Baer (1936) found specimens of

Received for publication, July 12, 1952.

* Authors ascribe the species to Davaine, 1869, but I have found no description of *T. madagascariensis* in that year and the species was described as a new species by Davaine (1870).

Raillietina in *R. rattus* of Madagascar and stated that *D. madagascariensis* of various authors comprises several species.

Human infection in the Western Hemisphere was first reported by Daniels (1895) who described two specimens, without scolices, as a new species, *Taenia demerariensis*. The worms were identified by Blanchard as *T. madagascariensis*, (according to López-Neyra, 1931), and they have since been studied by Joyeux and Baer (1929), López-Neyra (1931) and Dollfus (1940). Joyeux and Baer (1929) transferred the species to *Raillietina* and in the genus they recognized three groups of species reported from man: (1) worms from Madagascar and neighboring islands; (2) worms from the Far East; and (3) worms from South America. López-Neyra (1931) regarded *R. demerariensis* as specifically distinct from *R. madagascariensis*. León (1938) described *R. quitensis* from man in Quito, Ecuador. Material was sent to Professor Brumpt at Paris and studied by Doctor Dollfus. In a preliminary report, Dollfus (1939) described four new species and in a more complete account, Dollfus (1940) recognized eight species of *Raillietina* in material from man collected in South America and Cuba. Joyeux and Baer (1940) studied the original specimens of *R. quitensis* and concluded that these worms and those described in the preliminary report of Dollfus (1939) all were actually members of a single species, *R. demerariensis*. They reaffirmed the opinion, expressed in 1929, that there are three groups of species in the genus *Raillietina*, although they noted that it is difficult to distinguish between those from the Madagascar area and from the Far East. They discussed the variation which occurs in a single species and the total absence of information concerning the genetic constitution of cestodes in which the life-cycle is complicated by alternation of generations and of hosts. Although both Dollfus (1940) and Joyeux and Baer (1940) noted that no species of *Raillietina* had been described from wild or domestic mammals of South America, the latter authors repeated their (1929) hypothesis concerning the possibility of human infection by cestodes of other mammals. Baer (1940) suggested that *R. demerariensis* is a recent human parasite, that it may have been derived from species of the Far East which are normally parasites of rodents but occasionally infect man, and that it may have been introduced into South America by the original human immigrants.

López-Neyra (1943) discussed the thirteen species which had been described from man. *Raillietina loechesalavezi* Dollfus, 1940 and *R. kouridovaldi* Dollfus, 1940 were referred to the genus *Inermicapsifer* and considered synonyms of *I. cubensis* (Kourí, 1939). These worms were originally described by Kourí and Doval (1938a, b), named *Raillietina cubensis* by Kourí (1939) and, on determination by J. G. Baer, transferred to *Inermicapsifer* by Kourí and Rappaport (1940a, b). Of the remaining species, López-Neyra recognized only four: three; *R. madagascariensis* (Davaine, 1870), *R. celebensis* (Janicki, 1902), and *R. formosana* (Akashi, 1916) from Ethiopian and Oriental regions; and *R. demerariensis* from the Neotropical region. He noted that *Raillietina celebensis* and *R. formosana* occur also in rodents, but the other species had been reported only from man.

During the past ten years, species of *Raillietina* have been found in non-human hosts in both North and South America. Chandler (1942) described *Raillietina (R.) bakeri* from the fox squirrel, *Sciurus niger rufiventer*, in southeast Texas, the first species of the subgenus to be reported from North American rodents. He

noted that the worms were very similar to *R. loechesalavezi,* a species described by Dollfus (1940) from a specimen from an 18-year old child in Cuba. Dollfus had observed the similarity of this species and *R. demerariensis* and in his key (p. 553) had distinguished between the two on the number of oviferous capsules, 75–80 in *R. loechesalavezi* and 120–150 in *R. demerariensis.* Pérez Vigueras (1943) described *Raillietina (R.) halli* from *Capromys pilorides,* a wild rodent in Cuba. Baylis (1947) described *Raillietina (R.) alouattae* from howler monkeys, *Alouatta macconnelli,* taken near Paramaribo, Surinam (Dutch Guiana). He reported that the specimens showed closer affinities to *R. demerariensis* than to old-world forms. He distinguished *R. alouattae* from *R. demerariensis* on the larger number of testes, smaller number of egg-capsules, and on the form and structure of the cirrus sac and cirrus. Joyeux and Baer (1949) identified as *R. demerariensis* two contracted specimens, one with scolex, from the howler monkey, *Alouatta seneculus,* in Surinam (Dutch Guiana). After describing the worms they stated: "En conclusion, nous sommes amenés à constater que *R. alouattae* et *R. demerariensis* sont des parasites normaux de Singes hurleurs et que la second de ces espèces—peut-être la première aussi? peut s'égarer chez l'Homme, tout comme en Extrême-Orient les espèces parasites des Rats se rencontrent occasionnellement chez l'Homme. Ajoutons enfin que ces deux espèces sont les seules du genre *Raillietina* à avoir été signalées chez des Mammifères de la région neotropicale."

A species of *Raillietina* from the lappe or paca, *Cuniculus paca* (syn. *Coelogenys* or *Agouti paca*) and from the agouti, *Dasyprocta agouti* in Trinidad, was described by Cameron and Reesal (1951) as *R. demerariensis* var. *trinitatae.* Differences between *R. demerariensis* Daniels, the species described from monkeys, and the worms from the wild rodents of Trinidad were presented in tabular form. Cameron and Reesal stated: "There seem to be no characteristics except size separating these four forms and there appears to be considerable variation in dimensions and numbers of hooks, testes, and egg capsules even within the tapeworm from the same host. It seems probable that all represent a single species, but pending further study of the problem and to prevent subsequent confusion, the writers' material is regarded as a new variety (var. *trinitatae*) of *R.* (*R. demerariensis*). There seems less doubt, however, that all these forms, if not identical, have at least a common origin." Cameron and Reesal pointed out that the hystricomorph rodents, which include *C. paca* and *D. agouti,* are chiefly South American in their distribution and have seen their greatest development on that continent. Of this group, only the two species named above occur on the island of Trinidad, B. W. I., just off the coast of Venezuela. Both species, however, are widely distributed on the South American continent. Cameron and Reesal concluded: "Our recovery of this tapeworm suggests strongly that in this case both monkeys and man are accidental, or at least, recent hosts and that, as is the case in the Old World, rodents are the natural hosts. While both murid and sciurid rodents occur in Trinidad and continental South America and we are largely ignorant of the nature of their endoparasites, the fact that two agouti and four lappes were found infected suggests that this parasite is indigenous to the hystricomorph rodents of South America and was not introduced into that continent in human beings."

The specimens from *Proechimys cayennensis* are very similar to and probably identical with those studied by Cameron and Reesal.

DESCRIPTION
(Figs. 1–4)

The material consists of five scolices with attached strobilae and several fragments, some of considerable length. The longest complete worm is about 100 mm. in length, and there are three pieces of strobila, 40 to 55 mm. in length, composed entirely of gravid proglottids. The worms vary much in size although all are regarded as members of a single species. The maximum width of the strobila is 2.72 mm. The largest gravid proglottid is 0.66 mm. long and 2.72 mm. wide, but there are other specimens, with gravid proglottids 0.60 mm. long and 0.60 mm. wide. In such worms the width of the strobila does not exceed 0.64 mm.

The scolices (Fig. 1) are roughly rectangular, measure 0.27 to 0.34 mm. in width and somewhat less in length, although the posterior end of the scolex is not sharply delimited. The rostellum, retracted in all specimens, measures 0.06–0.09 mm. in width and bears a double crown of alternating hooks. There are about 175 of these hooks which measure 0.011 mm. in length and only about 1 micron in width. The ends of the anterior row protrude only about 2 microns in front of those of the posterior row. The suckers measure 0.13 to 0.14 mm. in diameter. Their openings are 0.06 to 0.09 mm. in diameter and near the opening each bears an internal band of 8 to 10 rows of smaller hooks which measure about 5 microns in length.

The scolex, as noted, is continuous with an unsegmented neck region which may be as wide as the scolex, or when extended is about five-eighths the width of the scolex. In the latter instance, there is a region 1.64 mm. long to the first cellular aggregates that indicate proglottid formation and another 1.64 mm. to the first recognizable proglottid. In the specimen with a retracted neck, (Fig. 1) there is only 1 mm. to the first recognizable proglottid which is 0.009 mm. long and 0.34 mm. wide. In this specimen there are 74 proglottids in the next mm.; 32 proglottids in the next mm., and in the following mm. 15 proglottids each about 0.07 mm. long and 0.38 mm. wide; here the first cellular aggregates of the genital ducts are discernible. After another 120 proglottids the first testicular aggregates are recognizable; here the proglottids are 0.13–0.16 mm. long and 0.5 mm. wide. Mature proglottids vary in size from 0.196 mm. long and 0.52 mm. wide to 0.26 mm. long and 1.50 mm. wide. There are only about 50 to 60 mature proglottids in a strobila and in different specimens they are situated 20 to 30 mm. from the scolex.

The musculature of the strobila agrees with the accounts of Baylis (1947), and Cameron and Reesal (1951) for the same or closely related species. The excretory ducts agree in size and location with the description of Cameron and Reesal.

The genital pores (Fig. 2) are unilateral, sinistral, located about one-third to one-half of the length of the proglottid from its anterior border. There is a small, common atrium which may be tubular, as figured by Cameron and Reesal, or wide and shallow, into which the cirrus sac and vagina open. The opening of the vagina is posterior and ventral to that of the cirrus sac. The genital ducts pass between the dorsal and ventral excretory ducts. The cirrus sac is oval to pyriform to clavate and often curved with the concavity on the posterior aspect. It measures 0.12 to 0.18 mm. in length and 0.050 to 0.066 mm. in width; it is somewhat shorter and more oval when the cirrus is protruded. The cirrus is small, 0.008 to 0.010 mm. in width and two to three times as long as wide. The tip may be enlarged

slightly. In appearance it agrees with the cirrus figured by Dollfus (1940) in Ann. Parasit. 17: 439; fig. 18. The distal portion of the cirrus sac is tapered when the cirrus is retracted. The proximal portion of the cirrus sac contains a coiled portion of the vas deferens. Outside the cirrus sac, the vas deferens is narrow and coiled, and extends mediad, in the anterior region, to the middle of the proglottid. The number of testes is variable, from 26 to 46, with about twice as many on the aporal as on the poral side of the proglottid. They measure from 0.03 to 0.05 mm. in diameter and are flattened dorso-ventrally. They are absent in front of the genital ducts and often in the area behind the vitelline gland.

The distal portion of the vagina, for a length slightly exceeding that of the cirrus sac is 0.02 to 0.03 mm. in diameter. This portion has a strong muscular wall and is surrounded by glandular cells. The lumen, like the distal portion of the vas deferens, is lined with cuticula which bears very fine bristles that point outwardly. The remaining portion of the vagina is dilated but not uniformly, has a very thin wall, and contains spermatozoa. Both the cirrus sac and the distal portion of the vagina may persist in gravid proglottids.

The ovary (Fig. 2) is situated at or near the center of the proglottid. It has 10 to 15 lobes, and in fully mature proglottids varies in size from 0.15 by 0.10 mm. to 0.30 by 0.165 mm. It is oval, wider than long. The vitelline gland is compact, oval in shape, wider laterally, 0.07 to 0.1 mm. in width, situated behind the ovary. The shell gland is small, situated dorsally between the ovary and vitelline gland. No uterus was observed; the eggs become dispersed in the parenchyma and enclosed in capsules.

Gravid proglottids vary enormously in size (Figs. 3–4) and in the number of egg capsules which they contain. Proglottids 0.5 mm. long and 0.65 to 0.95 wide contain from 40 to 80 egg capsules; a proglottid 0.65 mm. long and 1.96 mm. wide contained 156 capsules and another 0.66 mm. long and 1.52 mm. wide contained 243 capsules. The capsules varied from 0.08 to 0.14 mm. in diameter. When formed they are larger, with thick walls, gelatinous in appearance; later the walls shrink, become thinner and have a more compact structure. Each capsule contained 2–12 eggs, oval to subglobular to polyhedral in shape and 0.025–0.031 mm. in diameter.

DISCUSSION

The specimens from *Proechimys cayennensis* agree closely with the descriptions of *R. demerariensis* Daniels and *R. demerariensis trinitatae* Cameron and Reesal. In certain respects, they are intermediate between them. The features in which the present specimens differ from these descriptions may be explained by normal variation within a species and on differences in treatment of material. The form and relative positions of suckers, of rostellum, of cirrus sac, and length and width of the strobila are influenced greatly by muscular contraction; the size and form of the genital structures vary with the state of reproductive activity; and the number of testes and of egg-capsules is not at all constant. Comparison of Figures 3 and 4 will provide evidence of this variation.

The specimens from *Alouatta seneculus,* identified by Joyeux and Baer (1949) as *R. demerariensis,* show much closer agreement with those from *A. macconnelli* described by Baylis (1947) as *R. alouattae* than they do to the descriptions of *R.*

demerariensis. Both are parasites of howler monkeys from the same area. The worms from the monkeys are larger than those from man and from rodents, although certain structures are comparable. The monkey parasites may represent a distinct species, but if the worms described by Joyeux and Baer (1949) are actually *R. demerariensis,* then *R. alouattae* should be suppressed as a synonym. As pointed out by Joyeux and Baer (1940), there is no precise basis for determining specificity in cestodes, and the same species under different physiological conditions, or in different hosts, may manifest striking morphological differences. Furthermore, these parasitic species, as a result of their complex life-cycles, hermaphroditism, and self-fertilization, may readily develop divergent strains adapted to particular host species. Under these circumstances, and in the absence of data from controlled experiments to determine the effects of different environmental conditions on development and adult structure, specific determination remains merely a matter of opinion. It is highly probable that the present specimens are *R. demerariensis,* that native rodents of South America are the natural hosts, and that infection of man and monkeys is accidental or incidental.

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EXPLANATION OF FIGURES

Figs. 3 and 4 drawn to the same scale

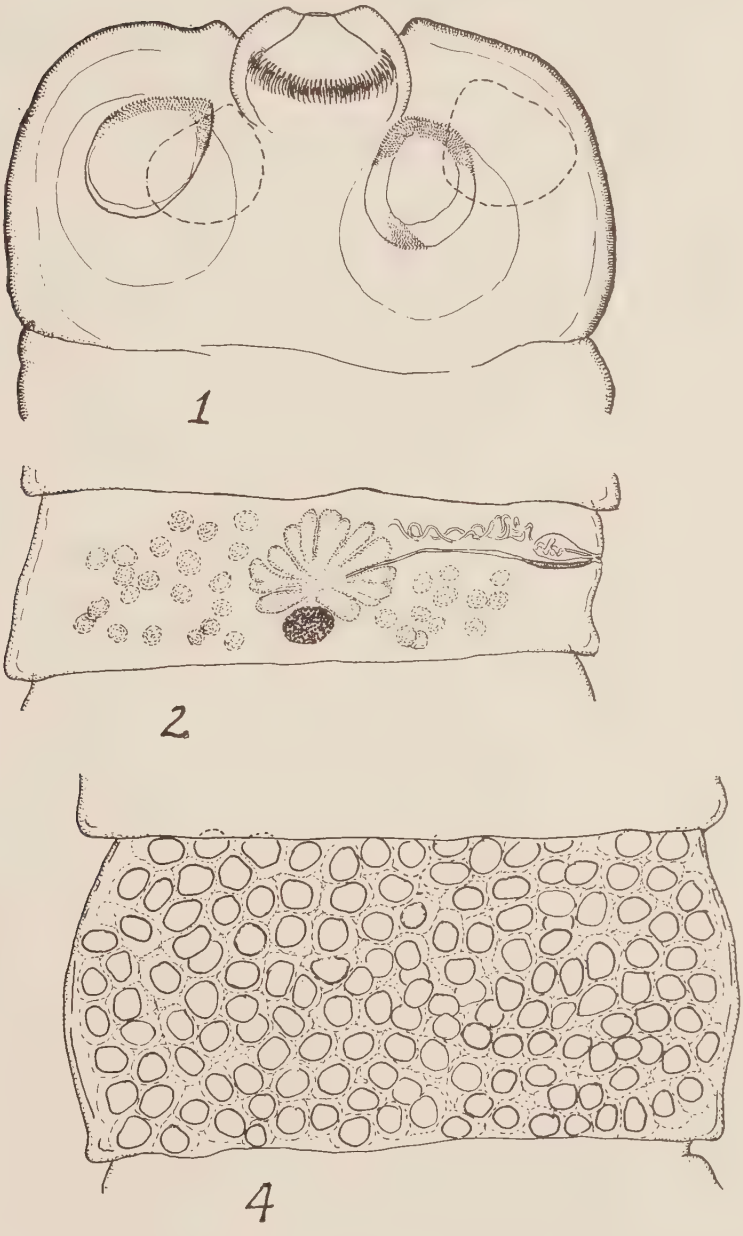
FIG. 1. Scolex, 0.3 mm. wide, of specimen with a wide, contracted neck.

FIG. 2. Mature proglottid, 1.33 mm. wide, excretory ducts omitted.

FIG. 3. Gravid proglottid, 0.6 mm. wide, from a small specimen; few egg-capsules with compact walls.

FIG. 4. Gravid proglottid, 2.52 mm. wide, from a large specimen; many egg-capsules with walls thicker, soft, gelatinous in appearance.

PLATE I



EXPERIMENTAL SCHISTOSOME DERMATITIS IN RABBITS

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Cercariae of certain species of schistosomes parasitic as adults in birds and small mammals can cause severe papular dermatitis in man. This subject has been reviewed by Cort (1950). Recently, Macfarlane (1949) and Olivier (1949) have shown that this is an allergic response which develops only after suitable sensitization. Other mammals may also develop dermatitis when exposed to these schistosomes. For instance, Augustine and Weller (1949) observed that in rabbits primary exposure was followed by no cutaneous reaction but that after repeated exposures there was a strong reaction with diffuse, flaccid edema and some crusting.

Vogel (1930), Brackett (1940), and Macfarlane (1949) observed that in the skin of sensitized persons there is a strong cellular response to the cercariae and the latter are apparently destroyed rapidly in the epidermis. Macfarlane concluded that in the skin of unsensitized persons there was a slow dissolution of the parasites in the epidermis with the formation of parakeratotic plaques.

On the other hand, Olivier (1949a, 1953) showed that following skin penetration of unsensitized mice, hamsters, guinea pigs, rabbits, and rhesus monkeys by avian schistosome cercariae, some of these worms migrated to the lungs of these animals. Penner (1941) showed that a schistosome of small mammals, *Schistosomatium douthitti,* could penetrate the skin of monkeys and migrate to the lungs. These observations demonstrated that although some of the schistosome cercariae that penetrate unsensitized laboratory animals may be destroyed in the skin, others leave the skin and reach the lungs. Furthermore, the observations on the migration of the schistosomes in monkeys suggest that migration of the same species to the lungs of man is a possibility.

In order to learn more concerning the fate of avian schistosomes in both sensitized and unsensitized animals, a study was made in rabbits of the skin reactions to these parasites. Rabbits were sensitized to schistosomes; skin biopsies were then made from these and from control rabbits after a challenge exposure to the same species of parasite.

Materials and Methods

New Zealand Giant white rabbits that had had no previous or intercurrent trematode infections were used. All cercariae were from snails collected in the vicinity of the University of Michigan Biological Station.[2] The cercariae were identified on the basis of morphology, activity, and snail host (Talbot, 1936). Cercariae used were those of *Trichobilharzia ocellata* (*Cercaria elvae*), shed by *Lymnaea stagnalis,* and of *Trichobilharzia stagnicolae,* shed by *Stagnicola emarginata angulata.* Both schistosome species normally develop into adults in birds (McMullen and Beaver 1945).

Received for publication, September 19, 1952.

[1] Laboratory of Tropical Diseases.

[2] The authors gratefully acknowledge the facilities which were extended to them at the University of Michigan Biological Station during the course of the study.

Routine exposures were made by placing freshly shed cercariae on the clipped, moistened skin in the inguinal region while the rabbit was restrained supine without anesthesia. For exposure of the areas to be biopsied, short glass cylinders with a 16 mm. bore were fastened to the clipped belly so that one end of the cylinder was in contact with the skin. Cercariae were then pipetted into the cylinders. This method exposed areas of limited size that could be clearly marked for biopsy and readily excised by simple surgical procedure. All exposures were for 30 minutes following which the water and glass cylinders were removed and the skin allowed to dry before the rabbits were taken from the restraining boards. Areas given the challenge exposures had had no previous exposure.

The skin biopsies were taken under intraperitoneal nembutal anesthesia. The full thickness of the skin was taken and the wound closed with interrupted silk sutures and painted with collodion to prevent removal of the stitches by the rabbit. Each biopsy was stretched gently on a cardboard and fixed in 10 per cent buffered formalin. A portion of each biopsy was sectioned serially and stained either with haematoxylin and eosin or a modified Romanowsky stain.[3].

Schistosome Dermatitis in Rabbits

Dermatitis produced by cercariae of Trichobilharzia ocellata

A 3-pound rabbit was exposed repeatedly to *T. ocellata;* it became sensitized as judged by the course of the successive reactions to exposure. It was first exposed on Sept. 9, 1948 to large numbers of cercariae in the inguinal region. The exposure was repeated 1, 5, and 11 days later. Following the first 3 exposures the skin developed macules and diffuse erythema which persisted for several days. These signs then disappeared and a distinct scaliness, which may have been dried exudate, was observed on the exposed skin. Eighteen hours after the last exposure the rabbit had approximately 50 pink papules 3 to 4 mm. in diameter in the exposed area. At 24 hours the papules were indurated and there was mild erythema, but no evidence of edema or pruritus. These papules persisted for 2 days.

Fifteen days after the first exposure this rabbit, and another of the same age, sex, size and breed which had not been exposed previously, were exposed simultaneously in 4 places on the belly to cercariae of *T. ocellata.* One of the exposed areas was then removed surgically from each rabbit 12, 22, 40 and 63 hours after exposure.

When the 12-hour biopsy was taken the exposed areas of the sensitized animal were edematous and red. Separate papules could not be distinguished. The control rabbit had only pale diffuse erythema in the exposed areas. At 22 hours the lesions in the three remaining areas of the sensitized rabbit were substantially the same as they had been at 12 hours. On the control rabbit there were numerous pink macules 1 to 1.5 mm. in diameter. At 40 hours the 2 remaining exposed areas on the sensitized rabbit had numerous discreet, hard papules 3 to 4 mm. in diameter surrounded by a zone of erythema. The control rabbit had numerous small macules only. At 63 hours the papules of the sensitized rabbit had disappeared leaving low, pale pink areas with crusty scales. On the control rabbit there were only a few pale macules.

[3] Grateful acknowledgment is made for the help of members of the Section on Pathological Anatomy of the National Institute of Arthritis and Metabolic Diseases who sectioned and stained the tissues.

Dermatitis produced by cercariae of Trichobilharzia stagnicolae

Five, 3-pound rabbits (numbers 1–5) were exposed repeatedly to cercariae of *T. stagnicolae* and all became sensitized as judged by their skin reactions.

The rabbits were first exposed on July 21, 1949 and exposure was repeated 1, 2, 4, 6, 8, and 11 days later. All exposures were followed immediately by the appearance of transitory macules or transitory erythema, usually the latter. Following the first 2 exposures there were no lesions after the disappearance of the macules or erythema. The third exposure was followed in 2 days by small papules with mild erythema. These papules persisted for several days. After the fourth and fifth exposures the skin became red and edematous after 48 hours and had many soft, confluent papules. Following the sixth exposure the reactions were similar to those just described though more severe. Three of the 5 rabbits developed moderate to severe dermatitis following the seventh exposure (rabbits 1, 2, 4). They had pronounced edema, erythema, induration and large confluent papules after 24 hours. The reactions faded rapidly and in 3 to 4 days were relatively inconspicuous. The other 2 rabbits (3, 5) had milder reactions with soft, less conspicuous papules and less erythema. In all cases the exposed areas became dry and scaly and the reactions waned.

Four days after the last sensitizing exposure, and 15 days after the first exposure, these 5 rabbits, and 3 suitable control rabbits (numbers 6, 7, 8) never before exposed to schistosomes, were given challenge exposures to the same schistosome species. One hundred cercariae were introduced into each of 4 glass cylinders fastened to the belly of each rabbit. In addition, water without cercariae was put in a fifth cylinder.

Six hours after exposure one of the areas exposed to cercariae, and also the control area, were excised and fixed. The 3 remaining areas were excised 12, 24, and 48 hours after exposure.

The course of the dermatitis in the 5 sensitized rabbits differed slightly from rabbit to rabbit but all developed papular dermatitis. Six hours after exposure all the sensitized rabbits had mild erythema in the exposed areas. After 12 hours 2 of the rabbits had distinct maculo-papules and the other 3 had mild erythema only. After 24 hours one rabbit (number 3) had mild erythema only while the other 4 had intense erythema, edema, and papules in the exposed areas. At 48 hours the remaining exposed area of all 5 rabbits had distinct papules usually accompanied by intense erythema and edema. Rabbits 1, 2, and 4 were judged to have had severe dermatitis while the other 2 had milder reactions. The control areas exposed only to water without cercariae had no visible lesions.

All 3 rabbits exposed for the first time to cercariae of *T. stagnicolae* had slight erythema in the exposed areas during the first 24 hours. At 48 hours one of them (number 8) showed no reaction and the other 2 had minute petechiae in the exposed areas.

Observations on Avian Schistosomes in the Skin of Sensitized and Unsensitized Rabbits

Trichobilharzia ocellata in rabbit skin

Portions of the biopsies from the two rabbits in the experiment with *T. ocellata* were sectioned serially and examined for worms. A total of 45 cercariae was found, 25 in biopsies from the sensitized animal and 20 from the control. In both animals,

most of the worms were in the epidermis, but some were in the corium. One worm was found in a hair follicle in each of the rabbits. Most of the worms found in the 12- and 22-hour biopsies were apparently alive, but all those found at 40 hours were apparently dead at the time the biopsy was made. Determination of the condition of the worms was difficult and was based on the following criteria: Worms considered alive had an intact cuticle, well-organized parenchyma and suckers, and apparently normal nuclei. Worms were considered dead if the nuclei were pycnotic, if the internal structures were abnormal, if the cuticle was conspicuously damaged, or if host cells had invaded the tissue of the parasite.

Trichobilharzia stagnicolae in rabbit skin

Serial sections from 30 biopsies were examined systematically and data on the location and condition of all worms found were recorded. A summary of these findings is presented in Table 1. A total of 196 worms was found, 122 in biopsies from sensitized animals and 74 from the controls. Most of the worms were found in the epidermis, but a few were in hair follicles, and a considerable number were in the corium. In the 24- and 48-hour biopsies from sensitized animals most of the worms were apparently dead when the biopsy was taken, but in the unsensitized animals most of the worms were judged to be alive at the time of biopsy. Figures 3–16 depict typical worms in the skin of both sensitized and unsensitized animals.

TABLE 1.—*Data from serially sectioned biopsies from 8 rabbits exposed to* Trichobilharzia stagnicolae

	Interval between exposure and biopsy (hrs.)	Number of biopsies	Number of serial sections studied	Total number of worms seen	Number of worms in epidermis	Number of worms in hair follicles	Number of worms in corium	Number of worms alive at time of biopsy	Number of worms probably dead at time of biopsy	Number of worms dead at time of biopsy
Five sensitized rabbits	6	5	730	25	24	0	1	18	6	1
	12	5	520	34	28	2	4	18	4	12
	24	5	695	40	31	7	2	8	7	25
	48	5	830	23	11	1	11	0	2	21
Three unsensitized rabbits	6	3	675	28	26	0	2	27	1	0
	12	1	100	8	7	0	1	8	0	0
	24	3	270	29	21	4	4	29	0	0
	48	3	365	9	1	0	8	3	4	2

THE TISSUE RESPONSE TO AVIAN SCHISTOSOME CERCARIAE IN THE SKIN OF SENSITIZED AND UNSENSITIZED RABBITS

In the skin of the unsensitized rabbits the cercariae aroused little or no cellular response. Worms in the epidermis lay in small cavities formed among the epithelial cells (Fig. 3). Although occasionally there was some cellular debris in the cavity with the worm there were no leucocytes or other cells. Similarly, the worms in the hair follicles and in the corium lay in apparently normal tissue (Figs. 1, 4, 5, 6) and most of them were alive at the time of biopsy.

On the other hand, the worms in the skin of sensitized rabbits aroused a conspicuous cellular response. Worms in the epidermis lay in tunnels and the space not occupied by the worm was filled with leucocytes and cellular debris (Figs. 2, 7, 11, 12, 15). In the 6- and 12-hour biopsies tissue cells had accumulated in the

corium near worms lying in the epidermis (Figs. 7, 11) and in the biopsies made later the number of these cells in the corium had increased greatly (Figs. 2, 12). In the biopsies from the rabbit sensitized to *T. ocellata* some of the worms in the epidermis lay in very large tunnels filled with leucocytes and the infiltration of cells into the corium near the parasites was very striking (Fig. 2).

However, the cellular response was greatest when the parasite lay in the corium. In the 6-hour biopsies the increase of cells about worms in the corium was small but recognizable (Fig. 8). In biopsies made later the cellular response to the invading worms was intense; the worms were always surrounded by a dense and usually extensive accumulation of cells (Figs. 9, 14, 15, 16). In the 22- and 24-hour biopsies the parasites in the corium could be recognized readily, but in the biopsies made later the worms were apparently disintegrating rapidly and could be recognized only with difficulty (Figs. 15, 16). Although some of the worms in the epidermis of 22- and 24-hour biopsies were probably alive at the time of biopsy, none of the worms in the corium of biopsies made after 12 hours was considered to be alive.

The cellular response in the skin of the rabbit sensitized to *T. ocellata* was much more intense at all stages than that in the skin of the rabbits sensitized to *T. stagnicolae*.

Specificity of Sensitivity to Avian Schistosomes in Rabbits

Olivier (1949) obtained strong cross reactions when persons sensitized to one species of avian schistosome were exposed to a second species. In order to get further information on this subject 3 rabbits sensitized to *T. stagnicolae* and 2 unsensitized rabbits were exposed simultaneously to cercariae of *T. stagnicolae* and *T. ocellata*. The intent of the experiment was to observe in the same hosts the reaction to the sensitizing species and to a closely related species.

The 3 sensitized rabbits (numbers 2, 3, and 4 of the series just described) and the 2 controls were exposed in 2 places on the shaved belly skin. Seventy-five cercariae of *T. stagnicolae* were placed on one of the areas and 75 *T. ocellata* on the other.

When the rings were removed the exposed areas on the 2 control rabbits showed no lesions. Rabbit 3 had mild erythema in the "ocellata" area and intense erythema in the "stagnicolae" area. In the other 2 sensitized rabbits the situation was reversed; there was more erythema in the "ocellata" area than in the "stagnicolae" area.

After 10 hours all the exposed areas on the sensitized rabbits showed erythema and edema. In rabbits 3 and 4 the reactions in the "stagnicolae" area were milder than in the "ocellata" area while in the other rabbit the situation was reversed. The control rabbits had either no lesions or very faint erythema.

After 24 hours there was a papular dermatitis with erythema in both exposed areas of all sensitized rabbits. In 2 of them the reaction in the "ocellata" area was more severe than that in the "stagnicola" area but this might have been caused by the penetration of more cercariae of *T. ocellata*. The control rabbits showed only mild erythema in the exposed areas.

After 48 hours there was no significant difference between the 2 reactions on the sensitized animals and all 3 rabbits had typical papular dermatitis in both areas.

DISCUSSION

It is apparent that rabbits can develop dermatitis following exposure to the cercariae of avian schistosomes and that they do so only after previous sensitization. The data presented here confirm and extend the observations of Augustine and Weller (1949) in this regard.

The course of schistosome dermatitis reactions in rabbits is comparable with that occurring in humans. In both instances macules give way in 12 to 24 hours to a papular dermatitis usually with extensive erythema and edema. The dermatitis in rabbits following sensitization to cercariae of *Trichobilharzia ocellata* did not differ from that in rabbits following sensitization to cercariae of *T. stagnicolae*. Moreover, rabbits sensitized to *T. stagnicolae* reacted as strongly to cercariae of *T. ocellata* as they did to cercariae of *T. stagnicolae*. Thus the reaction is not specific though it must be borne in mind that the two schistosome species are very closely related taxonomically.

Rabbits exposed to avian schistosomes for the first time never developed more than a very mild erythema in the exposed areas and sectioned biopsies from these areas contained worms both in the epidermis and in the corium that had attracted no cellular response. Most of the worms in these unsensitized animals were apparently alive even as late as 48 hours after penetration. Olivier (1953) showed that avian schistosomes can penetrate the skin and migrate to the lungs of unsensitized laboratory animals. Whether any of the worms seen in the biopsies from the unsensitized rabbits were capable of migration to the lungs cannot be determined but there seemed to be nothing to hold them in the skin. This is of interest especially in the light of Lindquist's finding (1950) that following primary exposure of hosts abnormal to the nematode, *Nippostrongylus muris,* an intense cellular reaction occurred in the skin.

Sectioned biopsies from the sensitized rabbits differed strikingly from those of the unsensitized rabbits. In the former, worms were found both in the epidermis and in the corium, and in every instance there was a noticeable cellular reaction to the worms. At 6 hours after penetration the reaction was mild; there was a slight increase in cells in the corium and the tunnels adjacent to worms in the epidermis contained many leucocytes. Later the reaction increased greatly in intensity and at 12 and 22 hours the reactions were relatively intense and some of the invading worms were apparently dead. At 24 and 48 hours the cellular response was even stronger and most of the worms were apparently dead. The response in sensitized animals is apparently intense enough to destroy at least some of the worms in the skin. The immobilization and destruction of the worms may be effected by humoral factors or by the cellular response or by both. In this connection it is noteworthy that Papirmeister and Bang (1948), Vogel and Minning (1949, 1949a), Liu and Bang (1950), and Standen (1952) have reported that when cercariae of *Schistosoma mansoni* are put in serum from schistosome-infected animals precipitates or membranes formed about the cercariae or the cercariae were agglutinated. These phenomena are apparently attributable to factors in the immune serum not found in normal serum.

Many worms were seen both in the epidermis and in the corium of rabbits, but since the study was not quantitative it is not possible to draw conclusions as to details of the history of the worms in the skin. Possibly some of the worms seen in

the epidermis of unsensitized rabbits may have been capable of migrating into the corium and on to the lungs. On the other hand, it may be that most of the worms still in the epidermis after 6 hours were destined to stay there and be sloughed off with the dying epithelial cells. Perhaps only those worms that penetrate into the corium quickly can migrate further. Worms were found in the corium as early as 6 hours after penetration; thus some worms, at least, reach the corium with little delay.

In skin biopsies from humans exposed to avian schistosomes (Vogel, 1930; Brackett, 1940; Macfarlane, 1949) worms have been reported only from the epidermis. The biopsies of Vogel and Brackett were taken from persons who had had previous exposure to schistosomes and in whom the reactions to the parasites in the skin may have been strong enough to prevent the cercariae from going farther than the epidermis. However, Macfarlane studied biopsies from 4 unsensitized persons and yet he also reported no worms from the corium. He implied that the cercariae in these cases did not go beyond the epidermis. The demonstration by Olivier (1953) that avian schistosomes can migrate to the lungs of laboratory animals, including monkeys, and the demonstration here that avian schistosomes may live in the epidermis and corium of rabbits for 2 days without exciting a cellular response suggest that this same situation may also occur in man and that cercariae of avian schistosomes are not necessarily destroyed in the skin of unsensitized humans. Further attempts should be made to determine whether avian schistosomes, and also other species of schistosomes not known to be parasites of man, may migrate to the lungs. Penner (1941) has already shown that *Schistosomatium douthitti,* a parasite of small mammals, migrated to the lungs of a rhesus monkey.

SUMMARY

Rabbits were sensitized to the avian schistosomes, *Trichobilharzia ocellata* and *Trichobilharzia stagnicolae* by repeated skin exposures to living cercariae. The course of sensitization and the resulting dermatitis in rabbits are comparable with sensitization and dermatitis in man.

Rabbits sensitized to cercariae of *T. ocellata* and *T. stagnicolae* were exposed to these same species and serial skin biopsies were taken from the exposed areas. Suitable control rabbits were also exposed and biopsied according to the same schedule. Sections of the biopsies revealed that the two species of cercariae penetrated the epidermis and corium of both sensitized and unsensitized rabbits. In the unsensitized rabbits there was no cellular response to the worms and all worms found appeared to be alive after 48 hours in the skin. In sensitized rabbits there was visible cellular response to the worms 6 hours after penetration and intense cellular reactions developed in 12 to 24 hours. Many of the worms in the skin of the sensitized rabbits were dead in biopsies made more than 6 hours after penetration. As a result of sensitization, the rabbits are apparently able to immobilize and destroy bird schistosome cercariae in the skin.

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Explanation of Plates

Plate I

Figs. 1 and 2. Selected portions of skin sections from rabbits exposed to *T. ocellata* (100×).

Fig. 1. Unsensitized rabbit exposed 22 hours previously. Note the worm in the corium and the absence of cellular response to the worm.

Fig. 2. Sensitized rabbit exposed 22 hours previously. Note the worm in the epidermis in a large space filled with leucocytes. Note also the heavy cellular response in the corium accompanied by edema.

Figs. 3 to 6. Portions of skin sections from unsensitized rabbits exposed to *T. stagnicolae* (130×). All the worms shown were probably alive at the time the biopsies were made.

Fig. 3. Portion of a 24-hour biopsy (rabbit No. 6). Note the worm in the epidermis without cellular reaction.

Fig. 4. Portion of 24-hour biopsy (rabbit No. 7). Note the worm in the corium next to the hair follicle.

Plate II

Fig. 5. Portion of a 24-hour biopsy (rabbit No. 7). There is a worm in the corium but no cellular reaction.

Fig. 6. Portion of a 48-hour biopsy (rabbit No. 6). There is a worm in the corium but no cellular reaction.

Figs. 7 to 16. Portions of skin sections from sensitized rabbits exposed to *T. stagnicolae*. Figs. 7, 8, 9, 12, 13, 14, 15, 16—130×. Figs. 10, 11—260×.

Fig. 7. Portion of a 6-hour biopsy (rabbit No. 2). Note the worm in the epidermis, the mass of leucocytes in the epidermis adjacent to the worm, and the increased number of cells in the corium.

Fig. 8. Portion of a 6-hour biopsy (rabbit No. 1). There is a worm in the corium and an accumulation of cells in the corium about it.

FIG. 9. Portion of a 12-hour biopsy (rabbit No. 2). There are two worms; the one on the left is probably in a hair follicle; the one on the right is in the corium. Both worms were probably dead at the time of biopsy.

FIG. 10. Enlarged portion of the serial section next to that shown in figure 14 showing the worm in the corium.

PLATE III

FIG. 11. Portion of a 12-hour biopsy (rabbit No. 5) showing a worm in the epidermis, leucocytes and cellular debris in the epithelium near the worm, and a mild cellular response in the corium. This worm was probably alive at the time of the biopsy.

FIG. 12. Portion of a 24-hour biopsy (rabbit No. 1). In the epidermis there is a worm that probably was alive at the time of biopsy.

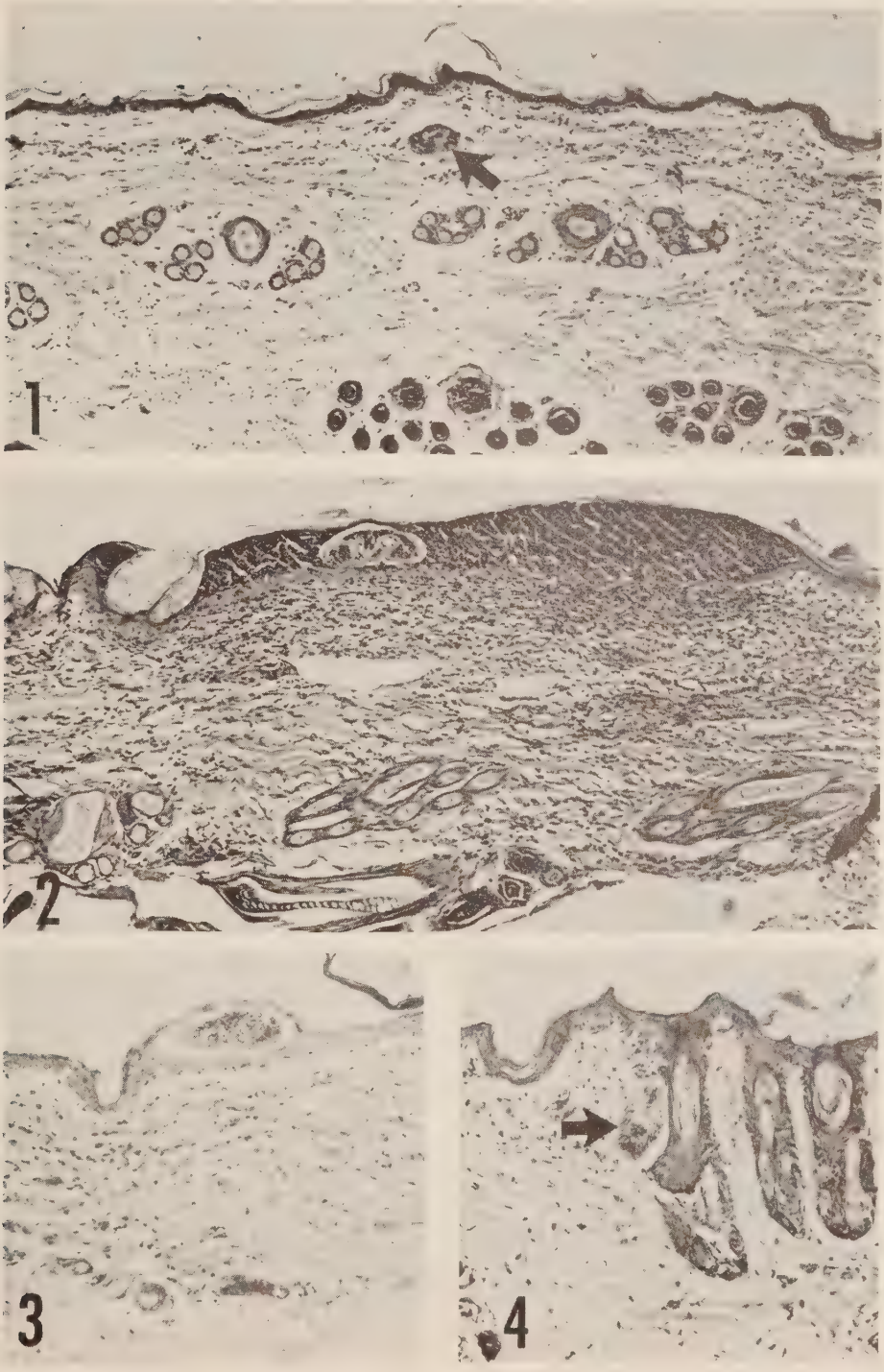
FIG. 13. Portion of a 24-hour biopsy (rabbit No. 5). The dead cercaria in the center of the figure probably lies in a hair follicle greatly distended by the cellular reaction.

FIG. 14. Portion of a 24-hour biopsy (rabbit No. 3). The arrow indicates a worm deep in the corium surrounded by an intense cellular reaction.

FIG. 15. Portion of a 48-hour biopsy (rabbit No. 5). The arrow indicates a dead worm in the corium. Note the extensive cellular reaction in the corium and the presence in the epidermis of a large mass of cellular debris. This is probably a sequel to the condition shown in Figure 2.

FIG. 16. Portion of a 48-hour biopsy (rabbit No. 2). Note the intense and extensive cellular reaction. The arrow indicates the probable remnants of a worm.

PLATE I



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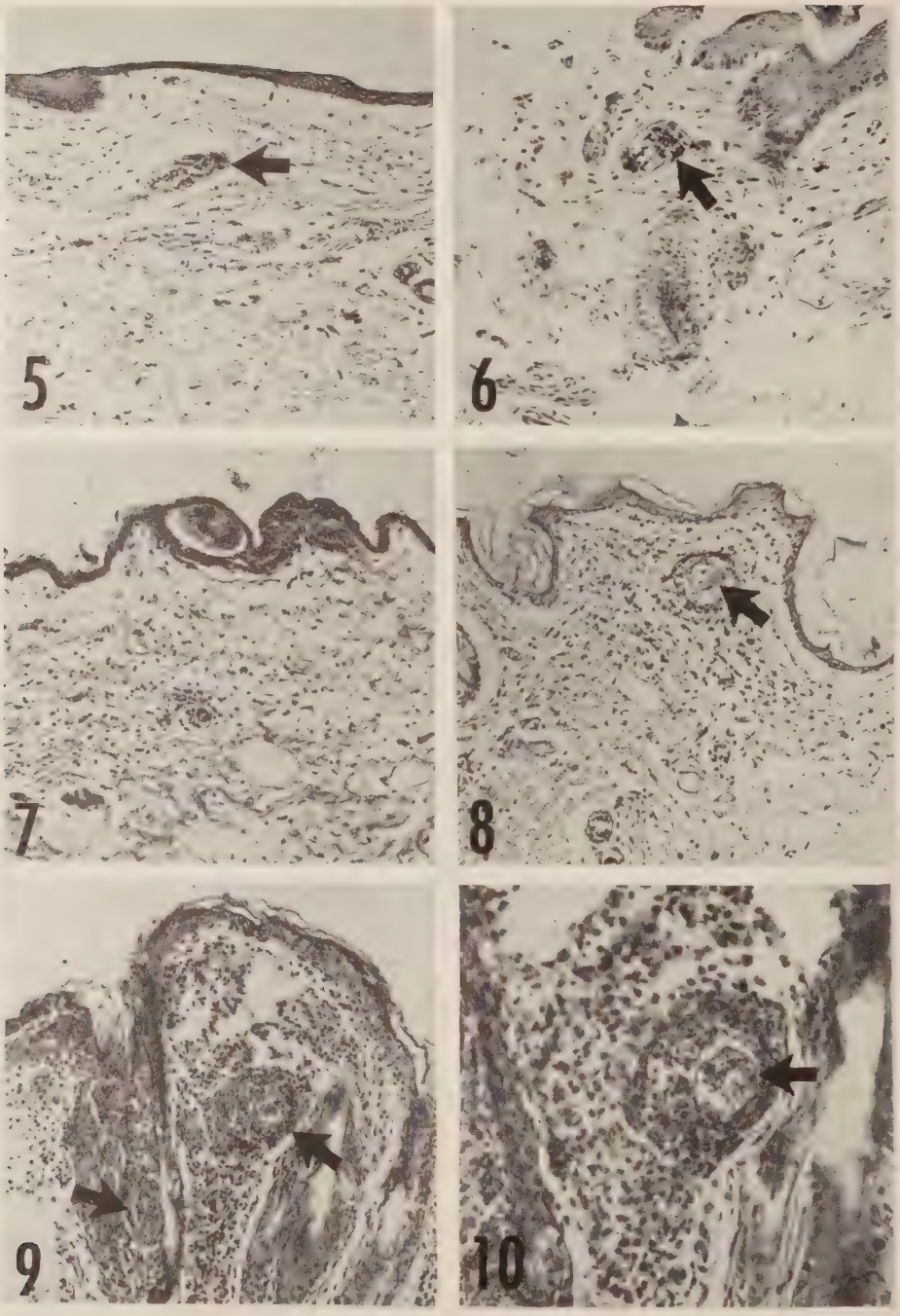
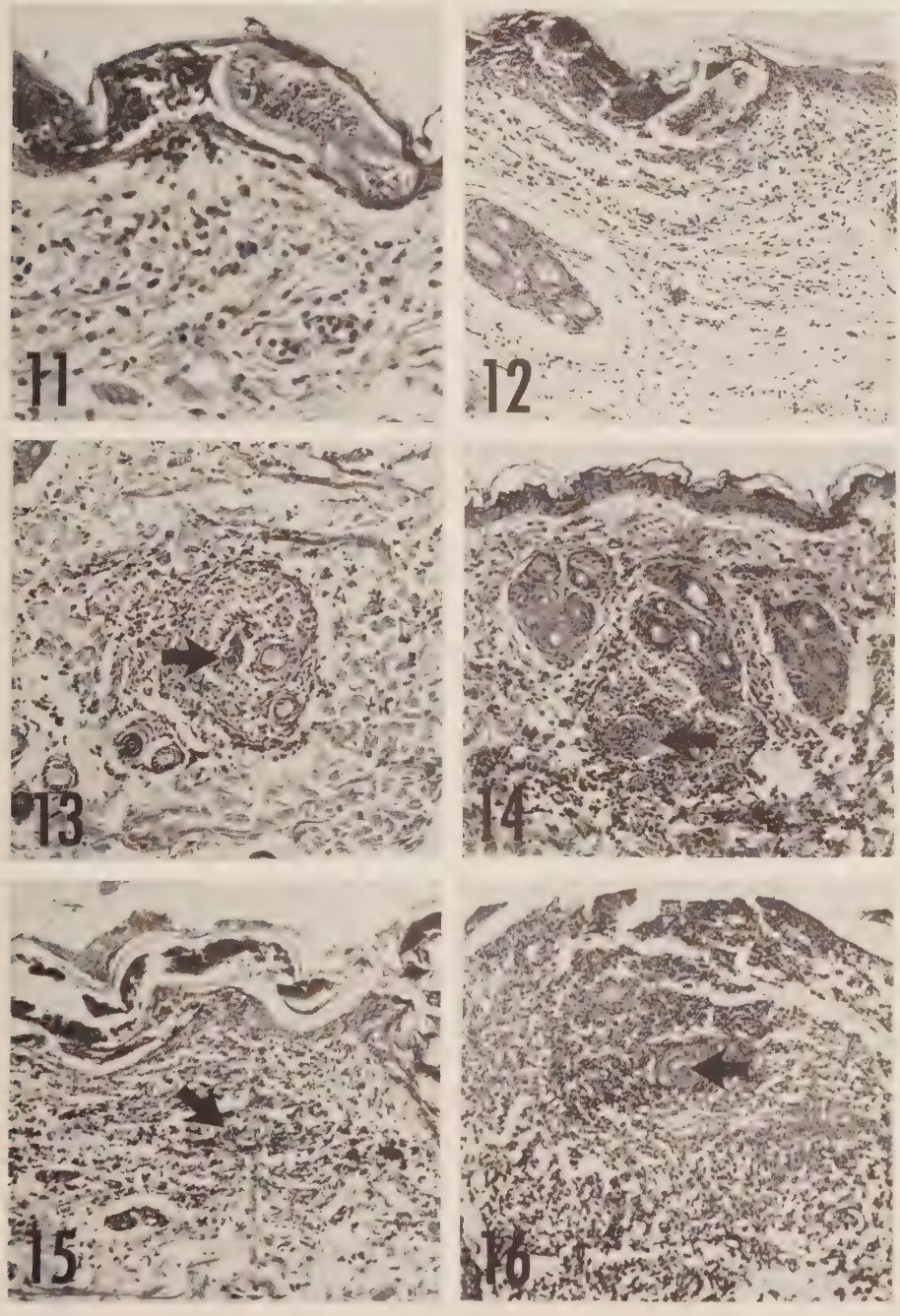


PLATE III



A NOTE ON THE CHIGGERS OF JAMAICA (ACARINA: TROMBICULIDAE)[1]

James M. Brennan[2]

The first published record of a trombiculid mite from Jamaica was by Ewing (1942) when he described *Schöngastia lynni* from a frog collected in the Blue Mountains. The description was based on four specimens, all with sensillae detached, a condition which led Ewing to express some doubt of the generic status of his species. Insofar as the present writer is aware, no additional specimens have been seen. The proposal of the new combination *Endotrombicula lynni* (Ewing) by Wharton and Fuller (1952) does, however, place the species in a more appropriate genus.

Six additional species including a new one from Jamaica are recorded. Of these, Jenkins (1949) previously reported *Trombicula batatas* and Brennan (1951) cursorily noted *Neoschöngastia americana* and *N. namrui.* These three records, derived from the material which is the basis of this paper, are repeated here for completeness. *Trombicula thompsoni,* the new species, is described below.

With the exception of *Whartonia nudosetosa* all collections were made by Dr. Gordon B. Thompson, Cambridge, England, formerly Associate Curator at the Institute of Jamaica, Science-Museum.

Trombicula thompsoni n. sp.

(Fig. 1)

Body: Length and width of holotype, partly engorged, 372 by 260 microns. Striae prominent. Eyes very large, 2/2, on an ocular plate. Anus located in posterior third of venter.

Gnathosoma: Cheliceral bases, capitular sternum, and palpal femoral and genual plates punctate. Blade of chelicera with tricuspid cap, the ventral tooth of which is much larger than the inconspicuous dorsal tooth. Palpal setae as follows: Coxal relatively short with long branches; femoral and genual branched; dorsal, lateral and ventral tibial nude. Palpal claw bifurcate, the axial prong inner and ventral. Palpal tarsus with 7 branched setae, an extremely long spur and a subterminala. Galeal seta nude.

Scutum: Roughly rectangular, densely punctate. Sensillae with few branches on apical half. Sensillary bases slightly anterior to level of posterolateral setae and separated by little more than the distance from either to the lateral margin. Scutal setae with appressed branches. Scutal measurements of holotype: AW—80, PW—89, SB—35, ASB—37, PSB—15, AP—32, AM—46, AL—49, PL—65, S—75.

Legs: All segments of all legs punctate. Setae distributed as follows: Leg I: coxa, trochanter and basifemur each with a branched seta; telofemur with 5 branched setae; genu with 4 branched setae, 3 genualae and a microgenuala; tibia with 8 branched setae, 2 tibialae and a microtibiala; tarsus with about 16 barnched setae, a spur, a microspur, a parasubterminala, a subterminala and a pretarsala. Leg II: coxa and trochanter each with a branched seta; basifemur with 2 branched setae; telofemur with 4 branched setae; genu with 3 banched setae and a genuala; tibia with 6 branched setae, a tibiala and a spur; tarsus with about 12 branched setae, a spur, a microspur and a pretarsala. Leg III: coxa and trochanter each with a branched seta; basifemur with 2 branched setae; telofemur with 3 branched setae; genu with 3 branched setae and a very long genuala; tibia with 6 branched setae and a very long tibiala; tarsus with 10 branched setae and a mastitarsala. All tarsi terminated by a pair of lateral claws with a slender claw-like empodium between.

Received for publication, October 20, 1952.

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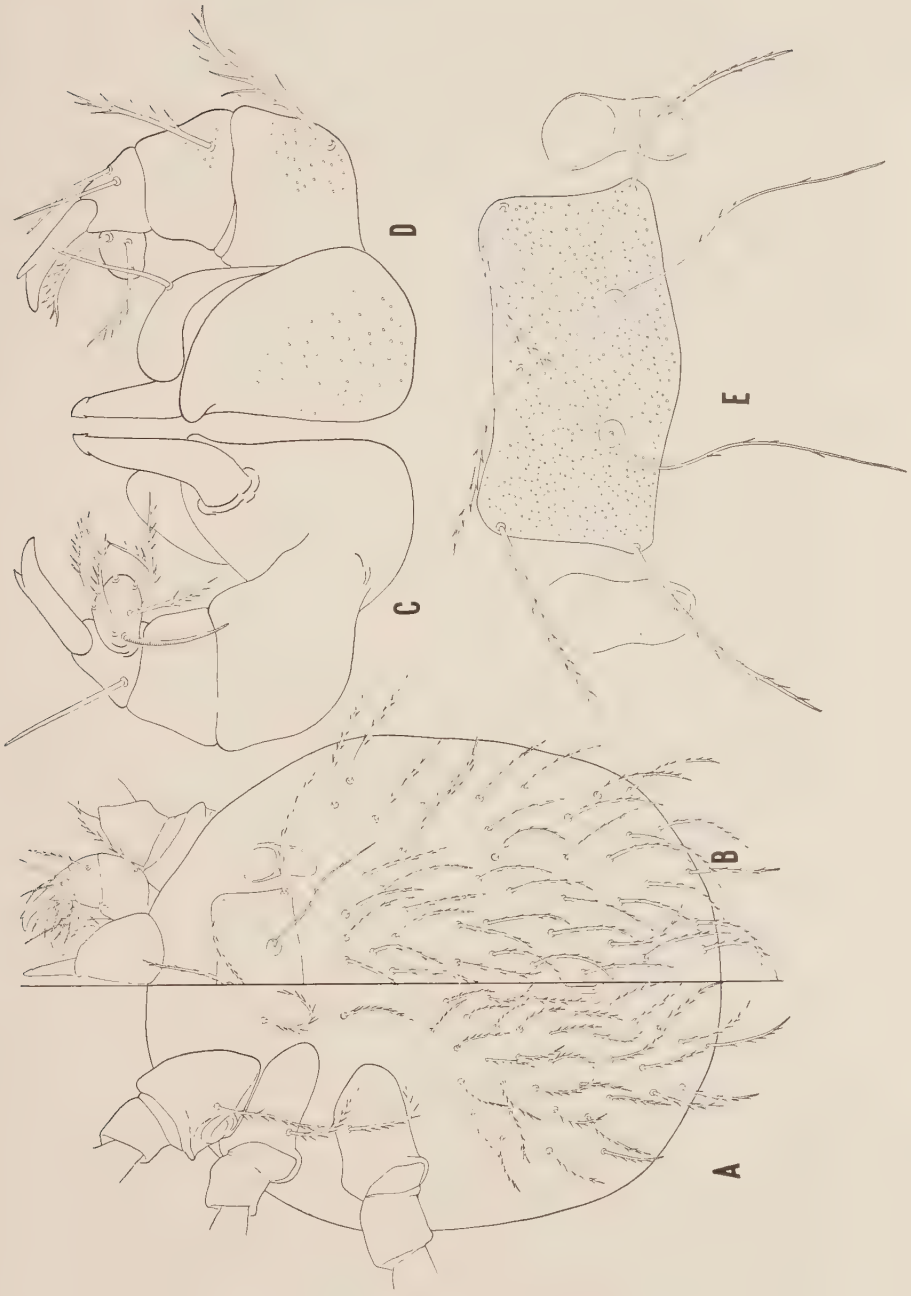

FIG. 1. *Trombicula thompsoni* n. sp. A. venter, B. dorsum, C. gnathosoma, ventral aspect, D, gnathosoma, dorsal aspect, E. scutum and eyes.

Setae: Dorsal setae in irregular rows, about 80, and a pair of humerals on each side. The setae are quite long, similar to the scutal setae, and do not exhibit any graded variation in length from anterior to posterior rows. Ventral setae 2–2 (sternals) plus about 80, those posterior to the anus similar to the dorsal setae.

Type data: Holotype, R.M.L. No. 23920, from *Arenaria interpres morinella* (ruddy turnstone); Jamaica, St. Andrew, Palisadoes; 28 March 1947; Gordon B. Thompson, collector. Deposited in the collection of the Rocky Mountain Laboratory.

Diagnosis: The bifurcate palpal tibial claw, the large number of dorsal setae and the presence of mastitarsala III in *Trombicula thompsoni* suggest affinities with *T. rioi* Gunther from which the former is readily distinguished by the branched genual seta and nude ventral tibial seta of the palp, the more slender accessory prong of the palpal claw, 2 humeral setae on each side, a greater number of dorsal setae, and a somewhat differently shaped scutum.

Remarks: The bird from which the single specimen of the description was taken was also infested with *Neoschöngastia namrui* Wharton and Hardcastle, recorded elsewhere in this paper.

Other species identified from Jamaica are recorded below along with their collecting data.

1. *Trombicula* (*Eutrombicula*) *batatas* (Linnaeus)
   Herpestes sp. (mongoose); St. Andrew, Kingston; 21 January and 15 March 1947; 7 specimens.
   Columbigallina passerina jamaicensis (ground dove); St. Andrew, Palisadoes; 16 Januaury 1947; 4 specimens.
2. *Trombicula* sp.
   Myadestes genibarbis solitarius (Jamaican solitaire); St. Thomas, Corn Puss Gap, 2000 feet; 14 November 1946; 2 specimens.

An unusually large chigger with one mastitarsala III, a deep, heavily punctate scutum with sensillae branched apically. The species is apparently undescribed, but the material at hand does not lend itself to adequate description, therefore must remain temporarily unnamed.

3. *Neoschöngastia americana* (Hirst)
   Dendroica discolor discolor (prairie warbler); Clarendon, Portland Ridge; 2 January 1947; 4 specimens.
   Saurothera vetula (lizard-cuckoo); Clarendon, Portland Ridge; 24 March 1947; 2 specimens.
   Mniotilta varia (black and white warbler); St. Thomas; 9 January 1947; 2 specimens.
4. *Neoschöngastia namrui* Wharton and Hardcastle
   Arenaria interpres morinella (ruddy turnstone); St. Andrew, Palisadoes; 28 March 1947; 17 specimens.

The occurrence of this bird chigger in Jamaica is of some interest since it has been known previously only from the type locality in the Western Pacific where it was recorded from the islands of Guam, Ulithi, and Okinawa.

5. *Whartonia nudosetosa* (Wharton)
   Leaf-nosed ? bat; Portland Point; 19 July 1942; C. B. Philip, Collector; 1 specimen.

SUMMARY

Seven species of chiggers are recognized from Jamaica. *Trombicula thompsoni* n.sp., herein described, *Trombicula batatas* (Linn.), *Trombicula* sp., *Endotrombicula lynni* (Ewing), *Neoschöngastia americana* (Hirst), *Neoschöngastia namrui* W. & H., and *Whartonia nudosetosa* (Wharton) are recorded along with their host and distributional data.

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ON THE PROBLEM OF NON-INFECTIVE MICROFILARIAE[1]

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Studies on the length of life of the microfilariae of *Dirofilaria immitis* and other filarial parasites have been accomplished by means of introducing the parasite into the blood of an uninfected host. The microfilariae of *D. repens* have been observed to survive for more than two and one half years after transfusion from an infected to an uninfected dog (Gruby and Delafond, 1852; Fülleborn, 1908, 1912) and, similarly, the embryos of *D. immitis* persisted for about the same length of time in the blood of the recipient (Underwood and Harwood, 1939).

In view of the above observations, it may be logical to question whether age of the microfilaria influences its ability to develop in the mosquito host. Preliminary observations on this problem were made during a study of *D. immitis* (Kartman, 1953) and are here recorded.

EXPERIMENTAL

A nine week old uninfected dog (R), obtained in Baltimore, was transfused with blood from another dog (D) showing about 30,000 microfilariae of *D. immitis* per cm.^{3} of blood. Thirty-five cm.^{3} of blood were removed from the recipient by way of the jugular vein and 45 cm.^{3} of infected blood from the donor transfused to the recipient. The infected blood was mixed with 2.5 per cent sodium citrate solution in proportions of 1 : 10. The recipient was also given one cm.^{3} of sodium pentobarbital and 0.75 cm.^{3} of metrazol during the course of the transfusion. The weight of the recipient was 3000 grams at the time of transfusion, and if one twelfth of the total body weight is blood then the recipient had 250 cm.^{3} of blood into which were injected 1,350,000 microfilariae. This would give a rate of 5400 microfilariae per cm.^{3} if we assume their even distribution in the circulation. Actually, only a small percentage of transfused microfilariae appear in the peripheral circulation (Hinman, *et al.*, 1934; Underwood and Harwood, 1939) since most of them are probably filtered out by the viscera, particularly the lungs (Fülleborn, 1908).

Six days after the transfusion a batch of *Anopheles quadrimaculatus* females was fed on the recipient and these were dissected fifteen days later for observations on the development of ingested filariae. Feedings with *A. quadrimaculatus* were thus made at various intervals until the mosquitoes failed to ingest any parasites. When this occurred, 5 cm.^{3} of the recipient's blood were haemolyzed with 2 per cent formalin and then spun at 2800 r.p.m. for ten minutes and the sediment searched for parasites. In addition, xenodiagnosis with *quadrimaculatus* females also was done. The resulting data from these experiments are shown in Table 1.

Received for publication, September 26, 1952.

[1] From the Department of Parasitology, The Johns Hopkins University, School of Hygiene & Public Health, Baltimore. The writer has to thank Drs. W. S. Bailey and J. H. Drudge for aid with the blood transfusion.

[2] U. S. Public Health Service, Hawaiian Field Station, Honokaa, Hawaii, T. H.

TABLE 1.—*Development of* Dirofilaria immitis *in* Anopheles quadrimaculatus *fed upon an uninfected dog with transfused microfilariae in its blood.*

Months after transfusion	No. females fed	Dog[1]	Larval stages recovered 15–16 days after infective meal		
			2nd	3rd	Encapsulated 3rd
1/5	23	R	0	12	5
3/4	22	"	0	20	4
1	10	"	1	4	0
1 3/4	15	"	1	5	0
3	17	"	2	4	0
3 3/4	14	"	0	0	—
4	14	"	0	0	—
Control	10	D	680	14	0
Control	4	"	292	0	0

[1] R—recipient.
D—donor.

The encapsulated larvae shown in the table were mainly found in the Malpighian tubules of the mosquitoes, but two of them were found in the head and thorax, respectively, of two different mosquitoes. No encapsulation of filarial larvae was ever encountered after infection of *quadrimaculatus* females from dog D.

Additional observations were made by testing the ability of newly formed microfilariae to develop in the mosquito. An adult female *D. immitis* was obtained at autopsy of a dog which had previously undergone a ten day treatment with arsenamide administered orally (Drudge, 1950). This female was quite active and appeared to be normal; its uterus was removed and macerated in dog serum, a drop of which then contained all embryonic stages of the parasite including many microfilariae which seemed no different morphologically from those in the dog's blood. This material was mixed with dog whole blood and fed to 10 *Anopheles quadrimaculatus* and 18 *Aedes aegypti* females through animal membranes about twenty minutes after removal of the adult worm from the dog. The serum and whole blood used were from uninfected dogs. The mosquitoes engorged upon the suspension of parasites and sample dissections showed active microfilariae in their midguts. Dissection of all mosquitoes fifteen days later gave completely negative results. Five control *quadrimaculatus,* fed on infected blood from the same dog, revealed normal development of the parasite.

A similar experiment was conducted with the frog filaria, *Foleyella brachyoptera.* Uteri from three adult worms were macerated in normal saline, mixed with uninfected frog whole blood, and then fed to two lots of *Aedes aegypti* consisting of seven and ten females, respectively. Lot #1, dissected twelve days later, showed one female with two infective larvae in its thorax and all other females negative. Lot #2 was dissected fifteen days later and showed one female with two infective larvae in abdomen and thorax, respectively, and another female with two unchanged microfilariae and one infective larva in its thorax. All other females were negative. Eight control *aegypti,* fed on infected frog blood, showed five to be infected with many larval stages in many parts of the haemocoele and labium fifteen days after their infectious blood meal.

DISCUSSION

It is quite probable that the adult parasites in the donor dog began reproducing about sixteen months prior to the transfusion since this host was two years old at

the time. Thus some of the microfilariae transfused into the uninfected recipient were not less than three months and possibly over twelve months old at the last successful feeding of *Anopheles quadrimaculatus*. There is no way of determining the actual age of the particular microfilariae ingested by the mosquitoes, but the experiment does suggest that *Dirofilaria immitis* microfilariae are capable of infecting a favorable mosquito host for at least three months after birth. Failure of the mosquitoes to ingest parasites beyond the three month period may be attributed both to the fact that microfilariae are filtered out of the peripheral circulation and that growth of the dog diluted the remaining circulating worms. At four and three quarter months after the transfusion the recipient's weight was 9500 grams, giving an approximate blood volume of 708 cm.^{3}.

The encapsulation of some infective *D. immitis* larvae in *A. quadrimaculatus* is of interest since it suggests that encapsulation may in part be determined by factors in the parasite. However, no explanation can be offered at this time for the fact that only the transfused parasites succumbed to encapsulation and that the phenomenon appeared to be quite transient.

The results obtained with newly formed microfilariae of *D. immitis* and *F. brachyoptera* suggest that these parasites *in utero* or recently shed may undergo a period of aging before they are as infective to mosquitoes as are the microfilariae found in the blood. This aging interval may be quite short in view of the finding that some *F. brachyoptera* microfilariae, from the female uterus, matured in the mosquito.

Bertram (1950) has suggested that loss of infectivity to mites, *Bdellonyssus bacoti*, of *Litomosoides carinii* microfilariae from infected cotton rats is due to the parasite's interaction with antibody the titre of which is dependent upon the female parasite's reproductive activity. On the other hand, if newly born microfilariae must undergo a period of aging before their maximum infectivity for the arthropod host is established, then the infectivity of the microfilariae in the blood at any time may depend upon the percentage of new-born parasites composing the population available to the intermediate host. In this connection, it should be noted that Hinman (1937) has shown that a single female *D. immitis* may give rise to over 20,000 microfilariae in a twenty-four hour period. It seems clear, at any rate, that the factor of age cannot be discounted at the present time and that quantitative evidence is needed to account for the nature of non-infective microfilariae.

SUMMARY

1. *Anopheles quadrimaculatus* females, fed upon an uninfected dog transfused with *Dirofilaria immitis* microfilariae from an infected dog, showed normal filarial development up to three months subsequent to the transfusion.

2. Encapsulation of some infective larvae of *D. immitis* was noted in *A. quadrimaculatus* during the first month following the transfusion.

3. Failure of new born *D. immitis* and all but a few *Foleyella brachyoptera* microfilariae to develop in the mosquito suggests that these parasites may have to undergo a short aging period before they attain maximum infectivity for the mosquito host.

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IXODES VENEZUELENSIS, A NEW SPECIES OF TICK FROM VENEZUELA, WITH NOTES ON *IXODES MINOR* NEUMANN, 1902 (ACARINA: IXODIDAE)[1]

GLEN M. KOHLS

A single specimen of the new species here described was discovered among ticks collected by Dr. William Beebe in northern Venezuela in 1946. Additional specimens were found in collections made in the same general region during a plague survey in the summer of 1950 by Commander J. M. Amberson and Dr. E. Schwarz of the United States Naval Medical School.[2]

Ixodes venezuelensis n.sp.
Female
(Fig. 1, A–F)

Body: No unfed specimens are available. A specimen which appears to be fully engorged, or nearly so, is 5.84[3] long, exclusive of the capitulum, by 4.10 wide and is suboval in shape.

Capitulum Length, tips of palpi to tips of cornua, from 1.01 to 1.07; width of basis 0.44 to 0.48. Basis subtriangular, much prolonged anterior to the insertion of the palpi. Posterior margin between the cornua nearly straight. Surface of basis faintly crazed. Cornua prominent and bluntly rounded. Porose areas moderate in size, the interval between them about equal to their diameter. Palpi long and slender, length of articles 2+3 from 0.75 to 0.81, outer margin nearly straight, median margin curved. In ventral view, the basis is narrowed behind the auriculae; transverse sutural line present but faint. Auriculae as sharply pointed, curved horns directed downward and backward. Palpi flattened on their inner faces, article 1 with a small oval plate. Hypostome shaped as figured; denticles arranged 2/2 apically, then successively 3/3, 4/4, 3/3, and 2/2 to the base; length about 0.52.

Scutum: Length, 1.44 to 1.54; width, 0.94 to 1.01 (15 specimens measured). Long oval, widest in front of the middle. Scapulae pointed. Lateral carinae prominent and extending from the scapulae to near the posterior margin; less distinct in about the posterior third of the scutum. Cervical grooves as broad depressions beginning near the emargination and extending about half the length of the scutum. Punctations moderate in number and rather uniformly distributed.

Legs: Moderate in size and length. Hairs few. Coxa I with a moderately long, slender internal spur, no external spur; II with no spurs; III with a short external spur; IV with external spur absent or only faintly suggested. All coxae flattened and with salient posterior edges. Tarsi mildly humped subapically. Length of tarsus I, 0.62; metatarsus 0.37. Length of tarsus IV, 0.59; metatarsus, 0.36.

Spiracular plate. Suboval, longer axis transverse, length, 0.31; width 0.28.

Genital aperture. Between coxae IV.

Male unknown.

Nymph
(Fig. 1, G–L)

Capitulum. Length from tips of palpi to tips of cornua, 0.42; width, 0.24. Basis subtriangular, prolonged in front of the insertion of the palpi as in the female. Posterior margin between the cornua nearly straight. Cornua short, about as wide as long. Surface of basis crazed. Palpi long and slender; length articles 2 and 3 combined about 0.32. Lateral margin of palpi a little concave, median margin curved. In ventral view, basis is narrowed posterior to the

Received for publication, October 20, 1952.

[1] From the Federal Security Agency, Public Health Service, National Institutes of Health, National Microbiological Institute, Rocky Mountain Laboratory, Hamilton, Montana.

[2] The following specimens of *I. venezuelensis* from Colombia were found in collections sent by the Chicago Natural History Museum after this paper was submitted for publication: Five females from *Oryzomys caliginosus,* near Valdivia, Antioquia, June 17, 1950, P. Hershkovitz coll. (RML 31415); one female from *Nectomys alfari,* data as above, June 29, 1950 (RML 31418).

[3] All measurements are in millimeters.

auriculae and is broadly rounded behind. Auriculae as short, posteriorly directed spurs. Palpi flattened on their inner faces. Hypostome shaped as figured; denticles arranged 3/3 except for about the basal third where they are 2/2; lateral denticles much the largest; length about 0.21.

Scutum: Length 0.53 to 0.63; width, 0.53 to 0.59 (6 specimens measured). Scapulae pointed. Lateral carinae distinct and extending from the scapular areas to the posterolateral margins. Cervical grooves as shallow depressions which first converge, then diverge and terminate near the posterior end of the lateral carinae. Punctations few, scattered, more numerous in the posterior portion.

Legs: Coxa I with a moderately long internal spur and a shorter external spur. Coxa II with a short external spur, III with a minute external spur, IV with the external spur faint or absent. A few long, slender, hairs present on all coxae. Tarsi without humps. Length of tarsus I, 0.38; metatarsus, 0.15. Length of tarsus IV 0.31, metatarsus 0.20.

Spiracular plate. Suboval, longer axis transverse, length 0.16, width 0.14.

Holotype: Female, from the spiny pocket mouse, *Heteromys anomalus anomalus,* Campamento Rafael Rangel, Sierra Maestra, Aragua, Venezuela, August 12, 1950 (RML 30659).

Paratypes: Two females, data as above. Three females, same data, July 19, 1950 (RML 30657); one female, same data, July 18, 1950 (RML 30658); two females, same data, July 19, 1950 (RML 30664); one female, 1 nymph, same data, July 25, 1950 (RML 30662); two nymphs, same data, July 1950 (RML 30660); one female, same data (RML 30661); three females, one nymph, same data, August 2, 1950 (RML 28923); one female, 3 nymphs from the opossum, *Monodelphis brevicaudata palliolata,* July 25, 1950 (RML 28924); one female from *Heteromys anomalus,* Rancho Grande, Venezuela, August 13, 1946 (RML 23639).

Holotype and some of the paratypes deposited in the collection of the Rocky Mountain Laboratory. Other paratypes deposited in the United States National Museum and the British Museum (Natural History).

The female of the new species resembles *Ixodes minor* Neumann 1902, as yet known only from Guatemala, in some respects such as size, the anteriorly prolonged basis capituli, and the presence of auriculae and cornua. In the new species, however, the palpi are longer and narrower; the scutum is notably larger and the lateral carinae are conspicuous, the legs are longer and only coxa III has a definite external spur while in *I. minor* external spurs are present on all coxae. Furthermore, well fed females of *I. minor* are more narrowed anteriorly than those of the new species.

Females of *Ixodes montoyanus* Cooley 1944 of Colombia have the coxal spurs arranged as in *I. venezuelensis* but are quite different in other respects.

Until recently *I. minor* has been known only from the types ". . . un ♂ et une ♀ accouplés, pris sur un *Hesperomys* sp. ? au Guatemala, par J. Rodriguez" (Neumann, 1902)[4]. These were redescribed by Nuttall and Warburton (1911) who stated that the specimens were mounted as microscopic preparations and that their description of the species corroborated that of Neumann as far as the present condition of the specimens permitted. Through the courtesy of H. T. Dalmat, the writer has received two males, and two females, *in copula,* from a "rat" at Finca "Chalaball," Acatenango, Chimaltenango, Guatemala, collected June 26, 1951. These specimens agree in most particulars with descriptions and figures of *I. minor* and there is little question as to their identity. The principal discrepancy noted is in connection with the coxal spurs of the male. According to Neumann, and Nuttall and Warburton, the spurs on coxae II, III, and IV are situated toward the middle of the posterior border. The specimens at hand have the spurs in the usual position at the posterior external angle. It seems probable that examination of the types as microscopic preparations rather than as alcohol-preserved specimens may have resulted in an erroneous interpretation of the position of these spurs.

[4] The female reported by Fairchild (1943) from Panama is actually an *Ixodes* nymph, species not determinable. It is not *I. venezuelensis.*

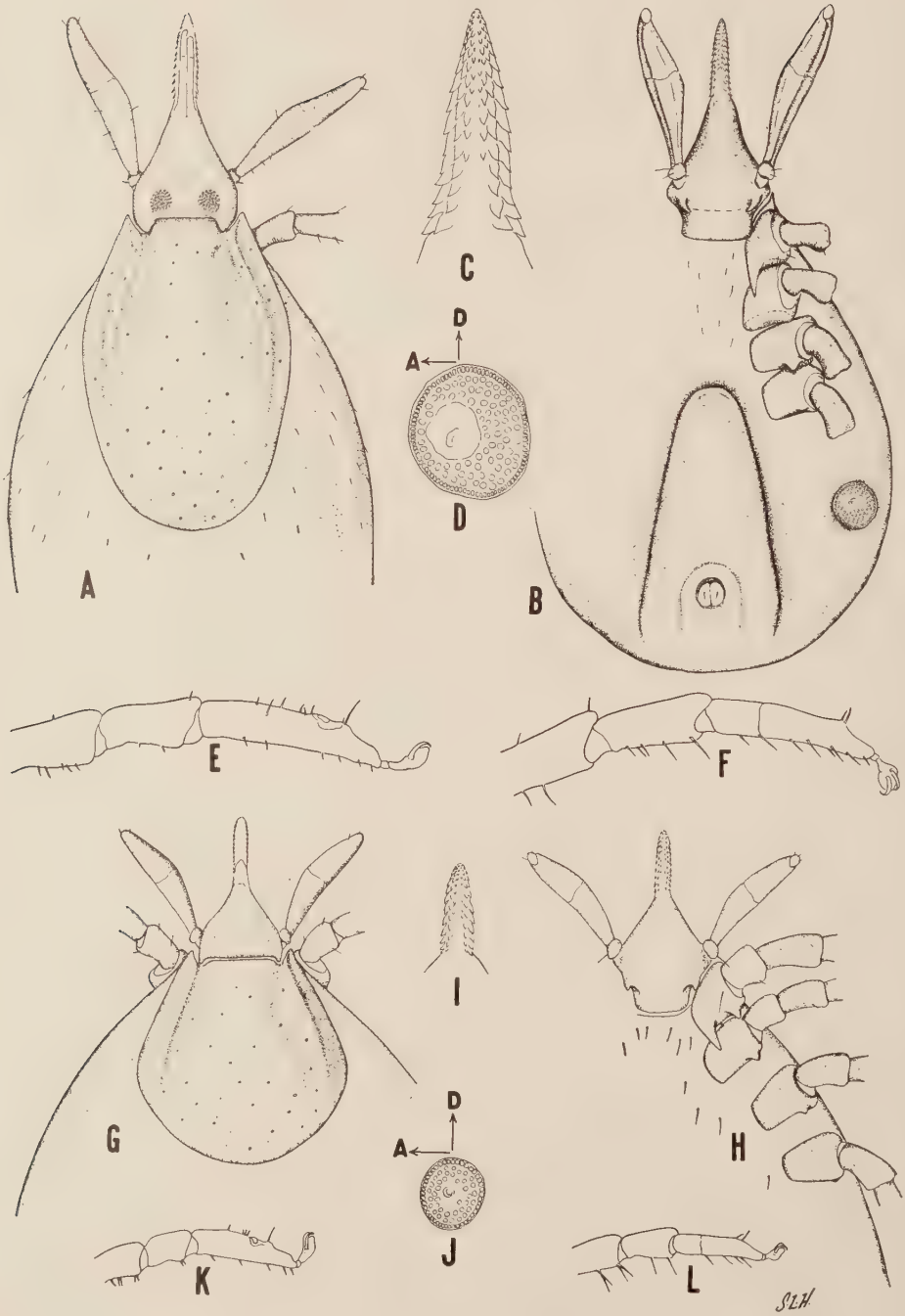

FIG. 1. *Ixodes venezuelensis* n.sp. A. Female capitulum and scutum, dorsum. B. Female capitulum and body venter. C. Female hypostome. D. Female spiracular plate (a=anterior, d=dorsal). E. Female metatarsus and tarsus, leg I. F. Female metatarsus and tarsus, leg IV. G. Nymph capitulum and scutum, dorsum. H. Nymph capitulum and coxae, venter. I. Nymph hypostome. J. Nymph spiracular plate. K. Nymph metatarsus and tarsus, leg I. L. Nymph metatarsus and tarsus, leg IV.

The body dimensions of the two males are length, 1.31 and 1.34, exclusive of the capitulum, width, 0.73 and 0.76, as compared with 1.45 by 0.8 in the type male according to Neumann, and Nuttall and Warburton. The hypostomes of the males agree well with that of type male as illustrated by Nuttall and Warburton. Neumann states that the scutum of the type female is 1.10 by 0.7, but Nuttall and Warburton state that the length is 1.3. The scutums of two females at hand are 1.09 and 1.12 in length by 0.75 and 0.78 in width. As in the type female, the hypostomes are broken off, and the entire capitulum is missing in one of the specimens.

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A NEW BLOOD FLUKE, *CARDICOLA LARUEI* N. G., N. SP., (APOROCOTYLIDAE) FROM MARINE FISHES[1]

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INTRODUCTION

During the summer of 1951 a series of white trout, *Cynoscion arenarius* Ginsburg, were examined for helminth parasites at Florida State University's Marine Laboratory, Alligator Harbor, Franklin County, Florida. Among the trematodes recovered were blood flukes belonging to the family Aporocotylidae. These flukes were again found in the same host and also in speckled trout, *C. nebulosus* (Cuv. and Val.), in the spring of 1952. These specimens could not be assigned to any existing genus or species. They were compared with and found to be congeneric with the type specimen of *Psettarium cardicolum* Manter, 1947, a species which Manter (1947) thought "might deserve generic rank" at the time he placed his species in the genus *Psettarium;* for these two species a new genus, *Cardicola,* is proposed. The present paper is concerned with a description of the new genus and a new species. A brief report on these worms is contained in a recent abstract (Short, 1952).

I wish to thank Mr. Paul C. White for his aid in collecting hosts and recovering many of the flukes. Sincere gratitude is also expressed to Dr. Emmett W. Price for the loan of type specimens of *Deontacylix ovalis* Linton, *Psettarium cardicolum* Manter and *P. tropicum* Manter from the Helminthological Collection of the U. S. National Museum, and to Dr. George R. LaRue and Mr. Allen McIntosh for reading the typescript of this paper and making valuable suggestions.

The first reported blood-fluke from fishes was described as *Aporocotyle simplex* by Odhner (1900) from flounders which were collected off the coast of Sweden. Odhner originally considered this species to be ectoparasitic on the gills, but later (1911) recognized it as a blood parasite. Soon thereafter Plehn (1905) described *Sanguinicola armata* and *S. inermis* from the circulatory system of European carp and tench. She first believed these flukes were parasitic turbellarians and subsequently (Plehn, 1908) considered them monozoic cestodes. *Deontacylix ovalis* was next described by Linton (1910) from a marine fish, *Kyphosus sectatrix* (L.), collected at Dry Tortugas, Florida, U.S.A. Linton apparently thought the worm came from the fish's intestine. It was Odhner (1911), however, who recognized Plehn's two species of *Sanguinicola* as digenetic trematodes. He indicated that Linton's *Deontacylix* was also apparently a blood fluke; and, noting the relationship of these blood flukes of fishes to each other, he proposed (Odhner, 1912), the family Aporocotylidae to include them. Stunkard (1923) considered it a strong possibility that *Deontacylix ovalis* had come originally from the mesenteric blood vessels of the host, and, in support of Odhner's opinion, assigned this fluke to the

Received for publication, November 10, 1952.

[1] Contribution from the Department of Zoology and the Marine Laboratory of the Oceanographic Institute of Florida State University.

family Aporocotylidae. The family now contains a total of 17 species in the following genera: *Aporocotyle* Odhner, 1900; *Sanguinicola* Plehn, 1905; *Deontacylix* Linton, 1910; *Psettarium* Goto and Ozaki, 1930; *Paradeontacylix* McIntosh, 1934 and *Plehniella* Szidat, 1951. Species of *Sanguinicola* and *Plehniella* have been recovered only from fresh water fishes, while all members of the other genera inhabit marine fishes.

MATERIALS AND METHODS

The flukes were dissected from the fishes' hearts in saline solution and were fixed in either A.F.A. (alcohol-formol-acetic) or Bouin's solution. Some specimens were fixed under slight coverslip pressure; others were pipetted into the fixative. Whole mounts were stained with Harris's hematoxylin, Semichon's aceto-carmine and Mayer's paracarmine. Frontal and transverse sections were stained with hematoxylin and eosin.

The following description is based on a study of 20 whole mounts, serial sections and a few living specimens. Unless otherwise stated, all measurements are in millimeters and are taken from whole mounts which were slightly flattened when fixed. The measurements given in the specific diagnosis are from the type specimen, with the ranges from paratypes in parentheses. Unless stated otherwise the measurements of ranges are from nine specimens.

OBSERVATIONS

Cardicola n. g.

Generic diagnosis. Aporocotylidae. Small, flat, elongate-oval trematodes, with margins of body recurved ventrally. Ventro-lateral body margins bearing short, transverse rows of small spines. Suckers absent; pharynx absent; oesophagus long, extending nearly to mid-region of body; intestine H-shaped. Testicular tissue in single mass lying between posterior intestinal ceca; single vas deferens. Ovary median, in posterior third of body; uterus relatively long, with few coils, postovarial. Genital pores separate, dorsal, postovarial; male pore posterior and lateral to female pore. Vitellaria extensive; unpaired median vitelline duct extending from region of esophagus to oviduct. In circulatory system of marine fishes.

Type species: Cardicola cardicola (Manter, 1947) n. comb.

Cardicola laruei n. sp.

Specific diagnosis: Cardicola. Body tapering about equally toward both ends from region near middle; length and width 1.357 (0.914–1.512) by 0.323 (0.222–0.356). Numerous tubercles on ventral surface of body (Figs. 4, 6). Spines on ventral margin of body arranged in about 600–670 short rows perpendicular to edge of body; rows of spines extending around body margin without interruption except for small area at anterior tip of body where mouth is located; number of spines per row usually from three to six, with numbers 4, 5, and 6 predominating; occasionally only two, or even a single spine representing a row. Spines delicate, elongated with sharp, curved tips (Fig. 7); approximately of same shape and size regardless of position; varying in length from 7.0 to 8.1 microns with average (10 spines) of 7.4 microns; deeply embedded, projecting about 3 microns beyond body surface with tips curved mesially (Fig. 4).

Mouth small, subterminal and ventral. Esophagus pursuing a slightly sinuous course almost to middle of body, length 0.685 (0.437–0.786); narrow near mouth, becoming gradually larger until width is 0.024 (0.016–0.034); posterior 1/5 to 1/4 of esophagus narrower than anterior part and of rather uniform diameter; wider anterior portion possessing hair-like projections on inner surface and surrounded by cells which are evidently glandular; narrower posterior part lacking gland cells and hair-like projections. Intestine H-shaped with two ceca extending anteriorly and two posteriorly from esophageal junction. Ceca not of equal length; usually anterior left and posterior right nearly equal in length and longer than other two. Measurements of lengths of intestinal diverticula (range from eight specimens): ant. right 0.316 (0.209–0.376), ant. left 0.382 (0.218–0.468), post. right 0.411 (0.269–0.499), post. left 0.307 (0.209–0.427).

Testis surrounded by definite membrane, length and width 0.253 (0.158–0.322) by 0.149 (0.073–0.161); bounded anteriorly by small group of vitelline follicles, posteriorly by ovary and laterally by posterior intestinal ceca; sometimes a few vitelline follicles occurring between lateral margins of testis and gut. Vas deferens well-developed, containing spermatoza, apparently serving as seminal vesicle; leading from a point near middle of posterior border of testis, crossing ovary ventrally, swinging toward left border of body, then mediad to midline of body and to left again where it opens through dorsal male pore which is situated on small papilla near left margin of body in region of lateral nerve cord; vas deferens doubling back on itself dorsally for a short distance about midway between its first and second arcs, and, before discharging to exterior, becoming constricted into short, narrow tube, then expanding into a small, bulbous terminal portion (Fig. 5). No seminal vesicle, cirrus or cirrus pouch observed.

Ovary more or less quadrangular with irregular margins, length in midline 0.076 (0.063–0.114), midpoint of one side to midpoint of other 0.120 (0.076–0.136); lying immediately posterior to testis, between posterior ends of intestinal ceca. Oviduct arising from posterior border of ovary at left of midline, extending toward left body margin along posterior border of ovary, bending sharply on itself and extending diagonally toward right body margin as far as right lateral nerve trunk, then looping dorsally and posteriorly to enter oötype; diagonal part of oviduct extending along posterior border of ovary enlarged, forming conspicuous, fusiform chamber for storage of spermatoza.

Oötype 0.157 (0.144–0.178; range from 7 specimens) from posterior end of body, surrounded by Mehlis' gland. Uterus entirely postovarial, extending from oötype posteriad a short distance, then anteriad with two loops to about level of midpoint of posterior margin of ovary, then turning sharply posteriad to open through female genital pore; terminal portion of uterus differentiated into short, muscular metraterm; uterus usually containing several thin-shelled eggs of rhomboidal, oval or spindle shape. Female genital pore dorsal, anteriomesial to male pore.

Vitellaria consisting of small follicles extending throughout most of body, from slightly anterior to nerve commissure to region behind ovary, and laterally beyond nerve trunks; follicles lacking in region of esophageal glands and forming thin layer ventral to testis and ovary. Main vitelline duct traceable in some specimens from mid-region of esophagus posteriad, ventral to testis, vas deferens, ovary and oviduct, to junction with oviduct near oötype; accessory ducts also occasionally visible joining main duct along its course.

Lateral nerve trunks conspicuous, joining posteriorly and united anteriorly by commissure dorsal to oesophagus.

Hosts: Cynoscion arenarius Ginsburg, the white trout (type host), and *C. nebulosus* (Cuv. and Val.), the speckled trout.

Location: Heart (one specimen from washings of gut of *C. nebulosus*).

Locality: Gulf of Mexico off Franklin and Wakulla Counties, Florida, U. S. A.

Type: Holotype and paratypes have been deposited in the Helminthological Collection of the U. S. National Museum, Nos. 37377 (type) 37378 and 37379 (paratypes).

Incidence of infection: Thirteen of 16 specimens of *Cynoscion arenarius* and 2 of 8 *C. nebulosus* were parasitized. The number of flukes ranged from 1 to 34 per host.

The species is named in honor of my professor, Dr. George R. La Rue.

DISCUSSION

As indicated above, the measurements given in the specific diagnosis are from specimens fixed under slight coverslip pressure. In specimens fixed without pressure the measurements are usually a little smaller. Also the margins of the body are curved ventrad, making the ventral surface concave (Figs. 2, 6). The position of the spines on the ventro-lateral body margins (Fig. 4) indicate that the spines are of importance in the maintenance of the fluke's position within the heart.

In such unflattened whole mounts, the spines appear to be more ventrally situated than they actually are because of the ventrad curling of the edges of the body. When a row of spines is viewed edgewise, the spines sometimes appear to be fused as Manter has stated they are in *Psettarium tropicum* (1940) and *P. cardicolum* (1947). Abundant material of *Cardicola laruei* was available for study, and it was determined that the spines are not fused. Even when rows of spines are viewed edgewise, careful focusing reveals several spines; and in unfixed specimens, the

spines can be dislodged by coverslip pressure and can be demonstrated to be definitely separate from each other. In these preparations individual spines appear as in Figure 7. In the specimens of *P. tropicum* and *P. cardicolum* which I have examined, the spines also appeared separate or at least not fused to the extent indicated by Manter. Actual counts gave the following number of rows of spines for five specimens of *Cardicola laruei:* 600, 633, 663, 665, and 669.

The new genus *Cardicola* is most closely related to the genera *Psettarium* Goto and Ozaki, 1930 and *Paradeontacylix* McIntosh, 1934. These three genera, all from marine fishes, are similar in general arrangement of the reproductive organs, and in the possession of relatively long postovarial uteri with postovarial male and female genital pores in similar relation to each other. The genus *Cardicola* differs from *Paradeontacylix* in having a single testis, whereas in *Paradeontacylix* the testes are numerous.

Psettarium, as well as *Cardicola,* has a single testis. However, in *Psettarium japonicum* (Goto and Ozaki, 1929) Goto and Ozaki, 1930, the type species of the genus, the testis is very extensive and conspicuously reticulate in nature, and the vitellaria are not follicular, but consist of "tubular acini" (Yamaguti, 1951). In addition to these differences, the ovary is multilobular and placed slightly to the right, and there are two vasa deferentia. It seems that these characters are of generic significance in separating *Cardicola* from *Psettarium.* More recently Manter (1940, 1947) described two flukes from marine fishes and (without the aid of Yamaguti's then unpublished 1951 paper) considered them to be congeneric with *P. japonicum,* naming them *Psettarium tropicum* (1940) and *P. cardicolum* (1947). In *P. tropicum* the testes were described as too indistinct and indefinite to allow counting, but the vitellaria are of small spherical masses and not tubular as in *P. japonicum.* The testis in *P. cardicolum* is a single mass almost completely filling the intercecal space from the intestinal bifurcation to the ovary, and does not extend laterally beyond the ceca (as does the testis in *P. japonicum*). The vitellaria consist of small follicles in this species also. Although the testes of *P. tropicum* and *P. cardicolum* are considered to be of the reticulate type, they are apparently not as reticulate in nature as in *P. japonicum,* and on the basis of the differences between the testes and vitellaria, it is my opinion that *P. cardicolum* (and possibly *P. tropicum* when its anatomy becomes better known) is not congeneric with *Psettarium japonicum.*

Manter (1947) considered that *P. cardicolum* possibly deserved generic rank, and I agree, especially in the light of Yamaguti's (1951) recent paper. Because of the close similarity between *Cardicola laruei* and Manter's *P. cardicolum* they are considered congeneric. *P. cardicolum* is designated the type of the new genus *Cardicola* and becomes *Cardicola cardicola* (Manter, 1947) n. comb.

C. cardicola differs from *C. laruei,* the only other species in the genus, in having relatively shorter anterior ceca, a bilobed ovary without a distinct membrane and no conspicuous diagonal sperm chamber in the oviduct. Other minor differences occur in the arrangement of the genital ducts.

Deontacylix Linton, 1910 differs from *Cardicola* in that the uterus of *Deontacylix* has preovarial coils, the testis is H-shaped with extracecal parts, and the ovary is located to the right of the midline.

Aporocotyle Odhner, 1900, the remaining genus from marine fishes, may be

separated from *Cardicola* by its possession of numerous testes as well as a preovarial uterus and genital pore.

Sanguinicola Plehn, 1905 and *Plehniella* Szidat, 1951 are both from fresh water fishes. Both genera further differ from *Cardicola* in that they have very short uteri and several testes, while in *Cardicola* the uterus is relatively long and the testis is one mass. The intestine of *Cardicola* also differs from that of *Sanguinicola* and *Plehniella*. *Cardicola* and *Sanguinicola* both have four ceca (except *S. chalmersi* which apparently has an irregular sac-like gut) but the ceca of *Cardicola* are relatively much longer. There are six short ceca in *Plehniella*.

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Explanation of Plate

Cardicola laruci

All Figures except Figure 7 were drawn with the aid of a camera lucida or microprojector. Scales are in millimeters.

Abbreviations: C intestinal cecum; D vitelline duct; E esophagus; EG esophageal glands; FP female genital pore; MP male genital pore; N nerve trunk; OD oviduct; OT oötype; OV ovary; T testis; U uterus; V vitellaria; VD vas deferens.

Fig. 1. Ventral view drawn from whole mount, slightly flattened.

Fig. 2. Ventral view of whole mount, not flattened.

Fig. 3. Posterior end of worm shown in Fig. 1, showing details of reproductive organs. Vitellaria omitted.

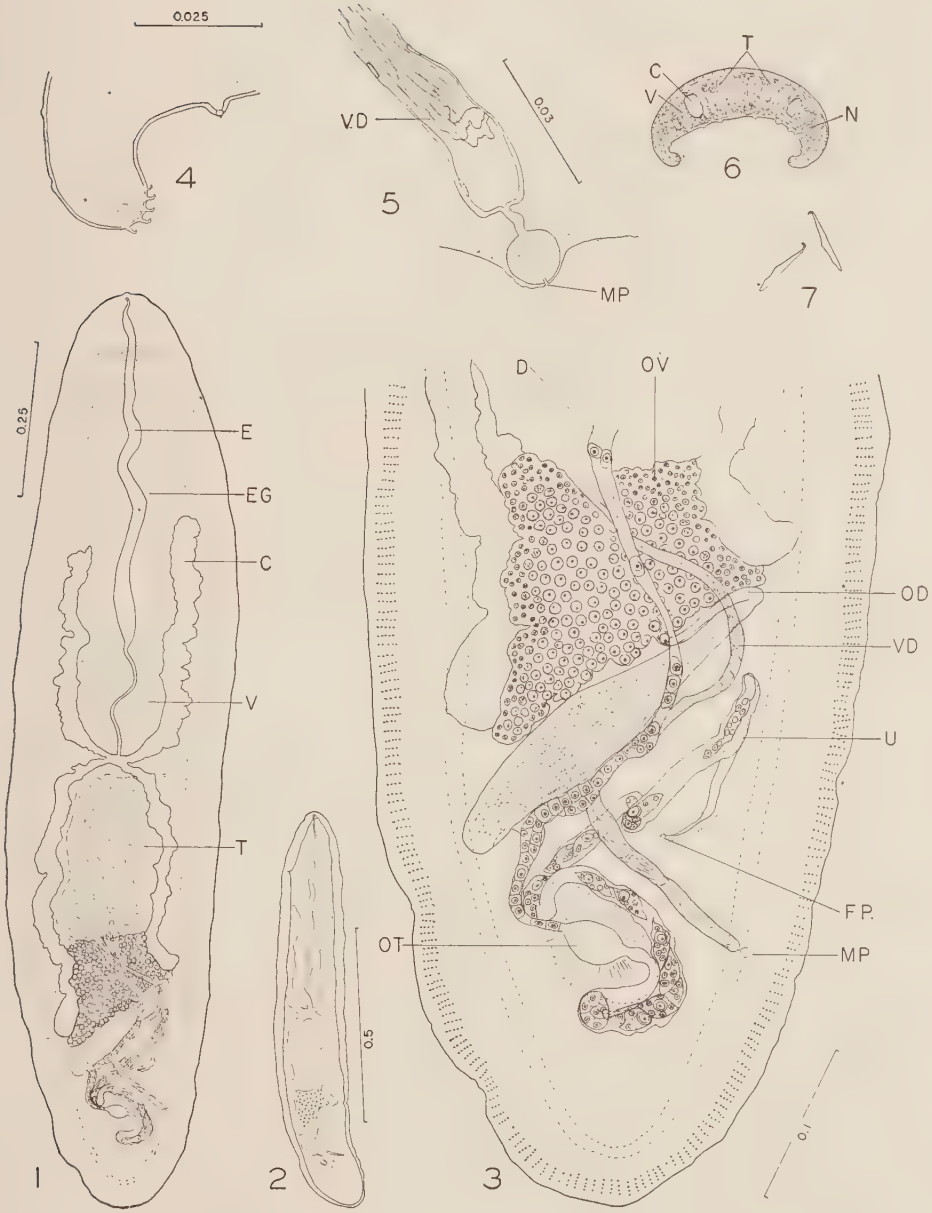
Fig. 4. Cross section of lateral margin of specimen, showing spines and one ventral tubercle.

Fig. 5. Terminal part of vas deferens and male genital pore. Drawn from sections.

Fig. 6. Cross section through testis.

Fig. 7. Spines removed from body, greatly enlarged.

PLATE I



THE LIFE HISTORY AND GERM CELL CYCLE OF *SPIRORCHIS ARTERICOLA* (WARD, 1921)*

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INTRODUCTION

Ward (1921) described *Spirorchis artericola* as the only trematode species parasitic in the vascular system of various fresh water turtles. He named the form *Proparorchis artericola* which became *Spirorchis artericola* when Stunkard (1921) suppressed the generic name *Proparorchis* as a synonym of *Spirorchis.*

The life history of *Spirorchis artericola* has not been determined. The present study is the first report of detailed observations on the germ cell cycle of any member of the family SPIRORCHIIDAE. The secondary germinal sacs in spirorchids are daughter sporocysts. All published studies employing both fixed and living material to determine nuclear phenomena as well as gross and cellular development in the germ cell cycle of digenetic trematodes concern forms in which the secondary sacs are rediae.

MATERIAL AND METHODS

Adult *Spirorchis artericola* were obtained by dissection and perfusion with citrated normal saline of the heart, arteries, and arterioles of the gut, pancreas, lungs, and mesenteries of painted turtles, *Chrysemys picta bellii,* from Swan Lake, a lentic basin covered with angiosperm vegetation, near Iowa City, Iowa, and *Chrysemys picta dorsalis* infected experimentally with cercariae from laboratory reared snails.

Eggs were freed from the feces of infected turtles. Some were studied and photographed, but most were incubated in well water until miracidia emerged. Over 1000 two- to thirty-day old snails, *Helisoma trivolvis,* reared from eggs deposited and hatched in the laboratory, were exposed individually to one miracidium each. Individual snails were examined immediately after penetration of the miracidium, one-half hour later, at twelve-hour intervals during the first three days, at daily intervals until cercariae emerged, at five-day intervals the following two weeks, and at irregular intervals during the three months following emergence of cercariae. In one series the infected snails were kept in well water in which the temperature ranged between 21° and 24° C.; in another series the temperature of the water varied between 24° and 28° C. Mature infected snails from Swan Lake were also studied.

Living miracidia, sporocysts, and cercariae in 0.5 per cent saline, quieted when necessary with 1 per cent chlorobutanol or vitally stained with 0.0005 per cent aqueous solutions of neutral red, Nile blue sulfate, or methyl green, were examined under phase and ordinary microscopes.

Received for publication, August 11, 1952.
* Contribution from the Department of Zoology, University of Iowa.
To Professor L. O. Nolf, under whose direction this investigation was conducted, the writer wishes to express her appreciation for his advice and encouragement during the course of the study.

Miracidia, fixed in 1 per cent silver nitrate, dehydrated in dioxan, and mounted in glycerine, were studied for epidermal plate pattern. Adult worms, entire juvenile snails, and the digestive glands of mature snails were embedded in paraffin and sectioned at six to eight microns. Whole mounts and sections of adult and larval stages were fixed in hot 10 per cent formalin, Carother's modification of Bouin's, Schaudinn's, or Flemming's fixatives; dehydrated in ethyl alcohol or cellosolve; and cleared in cedar oil or xylol. Whole mounts of all stages were stained in Grenacher's borax carmine or Delafield's hematoxylin and eosin. Stains used for sections included Delafield's hematoxylin and eosin, Heidenhain's iron hematoxylin without counterstain or counterstained with orange G or eosin, Goldman's (1949) modification of iron hematoxylin, or Flemming's triple stain.

Five eight-month *Chrysemys picta dorsalis* obtained from their nest were infected with cercariae which emerged from laboratory infected snails and three two-month *Pseudemys scripta* with cercariae from naturally infected snails. In both cases cercariae were added to the water in which the turtles were kept.

Drawings were made with the aid of a camera lucida. Drawings and photomicrographs were calibrated by means of a stage micrometer.

OBSERVATIONS

Life History

The adult worms were found in the heart, arteries, and arterioles of *Chrysemys picta bellii* and *C. picta dorsalis*. Eggs pass from the blood vessels into the surrounding tissue. Small areas of hemorrhage were noted surrounding some individual eggs and groups of eggs. Eggs were found in the muscular wall of the stomach and intestines, in the lungs, heart, mesentery, muscles, and pancreas, and but rarely in the liver and spleen. They pass to the outside of the body in mucous pellets in the feces. Eggs passed in the feces are spheroid, yellow to brown in color, and provided with an operculum which is not evident until it begins to open. Each contains a well-developed miracidium with eyespots. Fifteen eggs, after being in well water for twelve hours, measured from 0.116 to 0.145 mm. in length (average 0.128) and from 0.105 to 0.116 mm. in width (average 0.111).

The mature miracidium is visible through the transparent shell and vitelline membrane. It is surrounded by a transparent fluid. Several vitelline granules and a large globular structure, sometimes almost as large as the miracidium, are also present within the shell, but their position depends upon that of the miracidium. Under laboratory conditions, in which water temperature ranged from 22° to 28° C., hatching generally occurred on the second through the ninth day after deposition in the feces. Most of the eggs hatched either the first or the second morning after deposited in the feces. Although hatching usually occurred between 1:00 a.m. and 6:00 a.m., it occurred sometimes between 6:00 p.m. and 8:00 p.m. and, in several instances, between 1:00 p.m. and 3:00 p.m.. These latter times may have been due to unnatural conditions of lighting in the laboratory.

The miracidium is long, ciliated, bilaterally symmetrical, with a tapering anterior end. The body is widest at the level of the eyespots. Its size and shape vary greatly due to its power of contraction. Fifteen specimens fixed in hot 10 per cent formalin and prevented from flattening on the slide by means of coverslip supports measured from 0.122 to 0.147 mm. in length (average 0.133) and from 0.049 to 0.070 mm. in width (average 0.060).

The miracidia swim rapidly and occupy all depths of water. When the culture dish was placed so that there were areas of light and shadow, the miracidia congregated in the darker area. The body is covered with cilia, except for the anterior papilla and the spaces between the eighteen epidermal plates. These plates are arranged in four transverse tiers, containing six, six, four, and two plates in anterior to posterior direction. Cilia covering the anterior tier are shorter than those of the others. Inside the shell the miracidium contracts, so constrictions appear at the regions between the epidermal plates.

Beneath the epidermal plates a layer of cells covers the body. In the anterior region are located two large penetration glands, which sometimes extend past the middle of the body, and an apical gland. The neural mass is located near the center of the body, with a pair of pigmented eyespots near its anterior surface. In living specimens the eyespots have the form of an X or a small space between them, depending upon the amount of contraction of the body. An anterior and a posterior pair of flame cells connect to anterior and posterior collecting tubules. The tubules on each side unite and open to the outside in two excretory pores between the epidermal plates of the third tier. Eleven to fifteen large germinal cells occupy much of the posterior half of the body cavity.

The miracidia attacked and entered two- to twenty-day *Helisoma trivolvis*. Numbers of a species of *Physa* were exposed, but penetration occurred only in two instances in which the miracidia penetrated the mantle cavity. When these snails were examined twelve days later, no evidence of sporocysts could be found. Under the conditions of the observations, in which the miracidia were kept in well water changed twice daily, but not aerated, little penetration of snails occurred after 9:00 a.m. The miracidia began swimming more slowly as they became older, and attack of snails did not result in successful penetration.

Miracidia develop into mother sporocysts in the tissue of the snail. Mother sporocysts are long and sac-like with a rounded posterior and a tapering anterior end (Fig. 26).

Daughter sporocysts (Fig. 30) develop from the germinal cells in the mother sporocysts. In gross appearance they resemble mother sporocysts. Their number and rate of development vary according to the temperature of the environment and the size of the snail host. Eventually they may replace most of the host's digestive gland.

The cercariae which develop within the daughter sporocysts are apharyngeal, brevifurcate distomes. In lateral view the body is humped above the insertion of the tail stem (Fig. 24). There is no cuticular crest, but the furcae have fin-folds. Ten specimens fixed in hot 10 per cent formalin measured as follows: body length from 0.165 to 0.190 mm. (average 0.180), tail stem from body to tip of furcae from 0.588 to 0.640 mm. (average 0.615), length of furcae from 0.170 to 0.210 mm. (average 0.189), greatest width of body from 0.058 to 0.075 mm. (average 0.062), and greatest width of tail stem from 0.040 to 0.050 mm. (average 0.045). Small spines arranged in diagonal rows cover the surface of the body and tail. A ventral sucker is present on the ventral side of the posterior third of the body. The protrusible head organ is pyriform with the broad end anterior. Seven pairs of penetration glands are present in cercariae dissected from daughter sporocysts. A large, irregular mucin gland occupies the posterior humped region of the cercaria. Ducts

from the glands pass forward to the anterior end of the head organ where they open. The mouth is subterminal. The esophagus is long and tubular and bifurcates anterior to the ventral sucker into two short cecal pouches. There are six pairs of flame cells, five in the body and one in the anterior part of the tail stem. Four ciliated areas occur in the primary collecting tubules. There is no island of Cort. A pair of brown pigmented eyespots is dorsal to the bilobed ganglionic mass posterior to the head organ. In the ventral posterior region of the body a genital primordium is present.

Most of the cercariae produced in experimentally infected snails emerged between 6:00 p.m. and 9:00 p.m., but those from naturally infected snails emerged at various times in twenty-four hour cycles. Cercariae were observed to attack and penetrate turtles less than a year old of two species, *Chrysemys picta* and *Pseudemys scripta*. These were, however, the only species exposed. As the body of the cercaria enters the host, the tail is detached from the body.

Adult worms and eggs containing viable miracidia were recovered from *Chrysemys picta dorsalis* four months after exposure to the cercariae from laboratory infected snails. At this time masses of eggs were present in the lungs and walls of stomach and intestine.

Development and Reproduction in the Mother Sporocyst

During penetration into the snail the miracidium casts off its ciliated epidermal plates and becomes the mother sporocyst. Any exposed surface of the snail is penetrated. The time for penetration depends upon the area being penetrated and the vitality of the miracidium, but in these observations was between five and fifteen minutes. For a few minutes to several hours the miracidium-mother sporocyst migrates through the snail's tissues. It usually becomes fixed in the mantle, but may also become established in the foot or lymph spaces around the stomach or intestine.

When the temperature of the water surrounding the infected snails varied between 24° and 28° C., development was found to proceed as follows. The newly established mother sporocyst contains eleven to fifteen germinal cells, surrounded by somatic cells, in the posterior half of its body (Fig. 18). The somatic cells and any remaining eosinophilic granules of the penetration glands begin to disintegrate after the mother sporocyst becomes established in the snail (Fig. 19). By the third day very few remain (Figs. 20, 21). During the first three days little elongation of the mother sporocyst occurs. The newly penetrated sporocyst rounds up to form a spheroid or ovoid body. One fixed, dehydrated, stained, and mounted mother sporocyst measured 30 microns in diameter. A two-day mother sporocyst similarly treated measured approximately 35 microns in diameter and 50 microns in length (Fig. 19); a three-day one approximately 40 microns in diameter and 60 microns in length (Fig. 25); but a six-day sporocyst measured approximately 45 microns in diameter and 225 microns in length.

One-half hour after penetration of the miracidium into the snail the germinal cells of the parasite were observed to begin dividing. During the second through the fourth days the abundance of mitotic figures and the increase in the number of germinal cells in stained specimens indicated the rapidity of division (Figs. 19, 20, 21, 25). The sporocyst becomes filled with germinal cells. These resemble the

oocytes (Fig. 15a) of mature worms and may be recognized by their size, about 8 to 10 microns in diameter, their dense cytoplasm, the large nucleus, 6 to 7 microns in diameter, and the distinct cell membrane (Fig. 1). During the first two days of development the germinal cells become closely packed as division proceeds, but retain their spheroid shape. After the third day, however, some of the closely packed cells are nearly polyhedral. As the germinal cells fill the available space, the eosinophilic connective tissue decreases in relative abundance.

Between the sixtieth and seventy-second hour young daughter sporocysts begin to form. Germinal cells divide to form two unequal cells, a germinal cell, characterized by a large nucleus with knots of chromatin which lie along a fine network radiating from a deeply staining nucleolus and dense cytoplasm, and a slightly larger somatic cell, characterized by a more lightly staining nucleus and less dense cytoplasm (Figs. 4, 29). These cells correspond to the "propagatory" and "ectodermal" cells of Ishii (1934), Chen (1937), and others following the terminology introduced by Ishii. The somatic or "ectodermal" cell divides to form two smaller cells with granular nuclei containing nucleoli (Fig. 6). By the sixth day the mother sporocyst contains single germinal cells and groups of two to six cells (Fig. 26). Germinal balls made up of four to six cells have a single germinal or "propagatory" cell, cells with dense chromatin without nucleoli, and two sizes of cells with small nucleoli and less dense chromatin. The larger of the lightly staining cells becomes the spindle-shaped investing cell (Fig. 12a). Between the ten-cell (Fig. 11) and the thirty-cell stages the germinal cell has divided at least once to form two or more germinal cells like those in the miracidium-mother sporocyst (Fig. 14). In an eight-day mother sporocyst sixty-seven single germinal cells and four small daughter sporocysts were counted. The daughter sporocysts mature rapidly and escape from the mother. Transverse sections of a snail infected eleven days showed three daughters already in the digestive gland of the host. Most of the daughter sporocysts appeared to form simultaneously, but a few germinal cells and balls remained at least a month. The number of daughter sporocysts produced by one mother varies. For example, in one thirty-two day infection nineteen daughters of approximately the same size were counted; in another fifty-three day infection only five mature daughters were counted. These five filled the entire digestive gland of the host.

After the ninety-sixth hour of development the mother sporocyst elongates rapidly. From the second through the fourth day after penetration of the parasite into the intermediate host, some germinal cells appeared to form the body wall (Compare Fig. 18 with Figs. 19, 20, 21, 25, 26, 29). As elongation begins, the spheroid nuclei of the body wall cells become spindle-shaped; and, as elongation proceeds, the cells stain poorly and become squamous with small nuclei. The body wall of the two ends is thicker than in other regions. Greater elongation occurs posteriorly than anteriorly. The longest mother sporocyst dissected was from a snail infected twenty-four days and measured 0.63 mm. in length and 0.14 mm. in diameter in the living state.

Mother sporocysts dissected from snail tissue in 0.5 per cent saline solution moved feebly. As sporocysts older than nine days were freed from the mantle into the dissection fluid, constrictions appeared in the body wall. The expanded regions corresponded to the locations of the larger germinal balls and sporocysts. By the

ninth day spaces appeared between the developing daughter sporocysts (Fig. 28). Division of the excretory system occurs. In a nine-day mother sporocyst a large pair of flame cells was noted anterior to the eyespots and two pairs posterior. The eyespots remain at least three weeks, but are moved apart by elongation of the growing sporocyst.

Although evidence for maturation processes was carefully sought in the mother sporocyst, nothing resembling stages in meiosis was found. However, maturation-division figures (15b) were evident in the testes of mature worms examined for comparison. Nothing resembling trematode sperms (15c) could be found in sporocysts. Stages in mitosis were noticeable in sporocysts from twelve through ninety-six hours old (Figs. 16, 20, 21, 25). In some sections nearly all the germinal cells were undergoing mitosis. After the third day the germinal cells divided slowly, but a few remained singly or in loose groups throughout the life of the sporocyst.

Development and Reproduction in the Daughter Sporocyst

Early development of the daughter sporocyst parallels that of the mother sporocyst. Small germinal balls are present in the daughters before they migrate from the mother.

While the somatic cells divide repeatedly to form the body wall and connective tissue of the daughter, the germinal cell also undergoes mitotic division and produces a mass of germinal cells in the body cavity (Figs. 2, 27). For example, a stained and sectioned embryo comparable in size to a sixty-hour mother sporocyst was filled with large germinal cells in all stages of mitosis. When the daughter sporocysts begin to elongate, the germinal cells in the body cavity divide, but do not separate as before, and form a germinal and a somatic cell (Fig. 5). The somatic cell then divides to form two smaller daughter cells (Fig. 7). The cells of the somatic line divide to form the cells of several sizes characterized by nuclei containing small or no nucleoli found also in four- to six-cell daughter sporocysts (Fig. 9). Cell division of the developing cercaria (Fig. 10) resembled that of the developing daughter sporocyst, except that a larger number of deeply staining nuclei about 4 microns in diameter were noted in some preparations.

Development from the young germinal ball to that of the differentiated cercaria was more difficult to follow than in the young daughter sporocyst because of the lack of many germinal cells and tendency of all the cells to retain dyes. As the somatic cells begin to differentiate, the single germinal cell divides to form a few germinal cells (Fig. 13). After this, further development of germinal cells differs from that in the two previous generations. The germinal cells do not grow and multiply to form a mass of germinal cells filling the body cavity, but instead divide to form an aggregate of small, deeply staining cells, the genital primordium (Figs. 22, 23, 24). These cells do not differentiate further until the cercaria has escaped from the daughter sporocyst and penetrated a turtle, where distinctive cercarial characteristics are lost and adult ones formed.

In contrast to the more synchronous development of the daughter sporocysts, the cercariae develop in an anterior-posterior gradient (Fig. 30). As the cercariae develop they move freely in the body cavity and partly displace the germinal balls and cells. When daughter sporocysts were dissected from the host tissue, constrictions appeared in the body wall. These constrictions corresponded to the

spaces between the young embryos. The constrictions were less evident in daughter sporocysts supported by the glandular tissue of the host.

The first daughters escaping from the mother were noted on the digestive gland eleven days after infection. They appeared to travel along the lymph spaces to the digestive gland. In juvenile snails some daughter sporocysts were found in the mantle and lymph spaces around the stomach and intestine, but most of them were on the surface or in the digestive gland (Fig. 30). Eventually the whole digestive gland may be replaced by sporocysts.

Migrating daughters are elongated and bilaterally symmetrical with a spinose anterior end. The spines of the anterior end could not be found on mature daughters. During development within the mother, the body wall is first made up of spheroid cells which flatten (Figs. 28, 30). As the daughter sporocysts mature, the body wall becomes thin with irregularly placed small nuclei. The exact number of flame cells was not determined, but six were counted.

The number and length of the daughters and the number of cercariae developing depend in part upon the size of the snail and the consequent available space on and in the digestive gland. A 49-day, 1.5 mm. wide snail which was infected at two days contained some sporocysts having only two mature cercariae in them. The small snails contained an average of nine mature sporocysts. The longest daughter sporocyst dissected from a snail host was 5 mm. in length. It was really longer than that, for part of it was not freed from the entangled nearby sporocysts. This sporocyst was in a naturally infected snail 15 mm. wide. In this snail there were so many sporocysts which were entangled together that it was impossible to count them.

Rate of development of cercariae depends upon the temperature of the water in which the snails are kept. In the series in which the temperature of the water varied between 21° and 24° C., fifty-five days after penetration of the miracidium, daughter sporocysts contained cercarial embryos little beyond the germinal ball stage. All the available space on the surface of the digestive gland was covered with many sporocysts which averaged 0.215 mm. in length. When the parasitized snails were kept in water ranging from 24° to 28° C., cercariae emerged from the daughter sporocysts twenty-one days after infection.

Some infections seem to be terminated naturally, for the percentage of snails containing very young sporocysts was higher than that of snails containing mature cercariae. This appears to be due in part to the death of the host and in part to the death of the parasite. For example, a snail, from which cercariae emerged from the twenty-first to the forty-third day after penetration of the miracidium, was dissected the fifty-third day after infection. Part of the digestive gland was gone, but there were few traces of sporocysts or cercariae.

Single germinal cells occurring isolated or in loose masses remain in the body of daughter sporocysts (Figs. 3, 31). A snail 15 mm. wide was collected on September 28, 1950. Cercariae emerged daily from then until it died, October 24, 1950. Its digestive gland was almost obliterated by the mass of daughter sporocysts, which contained all stages of cercariae as well as single germinal cells. Another snail of the same size collected at the same time gave off cercariae until December 16, 1950, when it was dissected. It contained fewer sporocysts than the former and more digestive gland tissue. Some of the sporocysts were disintegrating.

The number of cercariae which develop from a single miracidium was not determined. A naturally infected, mature snail emitted 5 to 100 cercariae daily from October 16 to December 16. Even more cercariae were probably emitted from it, for it was not known when cercariae first emerged from it in nature. However, the infection in this snail may have been multiple. The digestive glands of two laboratory infected snails which emitted 0 to 5 cercariae daily for seventy-two days were filled with sporocysts containing both mature and developing cercariae when the snails were dissected ninety-three days after each was infected with one miracidium.

Although the exact number of chromosomes was not determined, the method by which cercariae were produced in the daughter sporocysts appears to be mitotic division of germinal cells (Figs. 17a, b, c) in the body cavity of the daughter sporocyst. Comparison of the metaphase plate of dividing cells in the young sporocyst with dividing somatic cells of the maturing cercariae showed similarity in number and placement of choromosomes. As in the development of the mother sporocyst, mitotic figures were most in evidence during the first week of development of daughters. In the development of cercariae from germinal cells of the daughter sporocyst, a comparable stage does not occur, for the few germinal cells divide to form the comparably small mass of cells which make up the genital primordium.

DISCUSSION

The life history of *Spirorchis artericola* resembles rather closely those for the previously described three species of SPIRORCHIIDAE (Wall, 1941a, b, 1951). Like the two species of *Spirorchis, S. parvus* and *S. elephantis,* the present species was found in *Chrysemis picta* and *Helisoma trivolvis.* The other spirorchid for which the life history is known, *Vasotrema robustum,* lives in a species of *Physa* and in the turtle, *Amyda spinifera.* The possibility that *Spirorchis artericola* may also live in other species of snails and turtles is not precluded, for that point was not determined in these experiments. However, when Ward (1921) named the species, he reported it in *Pseudemys elegans* from Havana, Illinois, in *Malaclemys pileata* from Newton, Texas, in *Pseudemys scripta* from Raleigh, North Carolina, and in *Chrysemis picta marginata* from Fairport, Iowa. It is probable, though, that Ward was dealing with more than one species of spirorchid, for he stated that certain specimens differed definitely from his account. Stunkard (1923) restricted the specific description of *Spirorchis artericola* to specimens collected in the heart and arteries of *Chrysemys picta marginata, C. picta picta,* and *Pseudemys scripta.* Byrd (1939) found *Spirorchis artericola* in *Pseudemys troosti, P. hieroglyphica, Chrysemys picta dorsalis,* and *Graptemys pseudogeographica pseudogeographica* from Reelfoot Lake in Tennessee.

The morphological differences among the SPIRORCHIIDAE thus far described are minor. The penetration glands of *Spirorchis artericola* are larger than in the species described by Wall, but resemble those figured by Stunkard (1923) for this species. The miracidium of *S. artericola* also differs from the species described by Wall in that hatching takes place most often between the twelfth and thirty-sixth hour after the eggs are passed in the feces. In the three species he studied, Wall found that hatching did not occur until the fourth day. Onorato and Stunkard (1931) also found that the spirorchid eggs they studied required five to seven days

for hatching. Ward (1921), however, found that hatching occurred in *S. artericola* four to twenty-four hours after deposition in the feces. Stunkard (1923) doubted whether this might be possible, for the conditions under which hatching occurred during Ward's observations were unnatural.

The gross morphology of the mother and daughter sporocysts described by Wall also resembles that of the species here described. Spirorchid mother sporocysts were found previously (Wall, 1941a, b, 1951) only in the mantle of the host; however, the reason why they were also found in the foot and lymph spaces around the stomach and intestine during the present observations may have been the larger number of specimens studied.

The morphology of the cercaria resembles that of other members of the family. Except for its smaller size, it resembles *Cercaria wardi* (Miller, H. M., 1923, 1926; Miller, E. L., 1936) which Wall (1941a) placed in synonymy with *Spirorchis parvus*.

The likenesses in the morphology of the larval generations of described SPIRORCHIIDAE leads one to the conclusion that if the life histories of the forty species named at present were known, they would not differ greatly from one another and that it is not possible to identify a spirorchid cercaria with any specific adult without experimental infection.

The observations recorded above provide a picture of development of larval generations by the process of simple polyembryony. The only spirorchid for which even a part of the germ cell cycle has been published is *Spirorchis elephantis,* which Brooks (1930) considered a schistosome. He stated, though, that the material did not offer as good an opportunity as might be desired to study the early phases of germ cells of sporocysts. He found cells which he called "ex-components" in the resting stage, "germ masses," germ balls, and cercariae in germinal sacs. No indication of ovaries and no maturation phenomena were observed. On the basis of the present study, the writer would call the "ex-components" germinal cells. Brooks called the cells which corresponded to the germinal cells of the present study "mature antecedent germ cells." He designated as "ex-components" the individual cells which were formed when a mass of components called a "germ mass" broke up.

According to the latest revision of the family SPIRORCHIIDAE (Byrd, 1939), the families most closely related to the spirorchids are the other families of blood flukes, the APOROCOTYLIDAE and the SCHISTOSOMATIDAE. No study of the germ cell cycle in the former family has been published. Brooks (1930) noted that the results recorded for *S. elephantis* were confirmed in *Trichobilharzia ocellata,* and in addition, "antecedent germ cells" were found free in young daughter sporocysts. In *Schistosomatium douthitti* (Cort, Ameel, and Olivier, 1944) and in *Schistosoma mansoni* (Olivier and Mao, 1949) several hundred germinal cells are produced within each mother sporocyst by multiplication of the germinal line carried by the miracidium. Each germinal cell gives rise to a daughter sporocyst embryo. Each daughter sporocyst produces cercariae mainly by means of division of germinal cells to produce a cercaria from each; however, some of the germinal cells give rise to germinal masses which in turn produce cercariae by secondary polyembryony. In *Trichobilharzia stagnicolae* Cort and Olivier (1943) showed that both daughter sporocysts and cercariae are produced by primary and secondary polyembryony involving germinal masses.

The germ cell cycle in *Spirorchis artericola* resembles that of the schistosomes more than it does that of any group studied thus far; however, no germinal masses were seen in the mother sporocyst, and the loosely connected groups of germinal cells in the daughter sporocysts appeared less definite than those described for the schistosomes. The cercariae of *S. artericola* also emerge from the daughter sporocyst through a terminal birth pore, but this structure was not seen in the mother sporocyst. The strands of connective tissue described in the schistosome sporocysts were not so evident in sporocysts of *S. artericola.*

Single germinal cells appear more often than groups in two- to four-month *S. artericola* daughter sporocysts. Since cercariae are formed from the germinal cells in an anterior-posterior gradient, germinal cells are located in loose aggregates in the posterior region of the daughters. When the cercariae begin to move about, the germinal cells become scattered among the developing germinal balls and mature cercariae. Some of them remain in the resting stage for at least several months; and, after the rapid development of cercariae in the young daughter sporocysts, a slow but constant development of germinal cells into cercariae may take place.

Although the number of cercariae emerging from a snail infected with a single spirorchid miracidium was not determined in this experiment, observations of others indicate that this may be in the thousands. Cort (1922) studied emergence of *Spirorchis elephantis* from *Helisoma trivolvis* during the summer of 1920 at Douglas Lake. The average daily number from one snail was 400 with approximately 12,400 for one month, a time which Cort believed did not represent the entire period of cercaria production, for the peak was at the beginning of the observations. Approximately 200 sporocysts were dissected from one snail. In a study of the emergence of *S. elephantis* from *Physa halei* in the Okoboji region in Iowa during the summers of 1934, 1935, and 1936, Heckwolf (1937) found that most of the snails produced 0 to 400 or 1000 to 5000 cercariae daily, but a few produced as many as 5000 to 15,000 daily. The maximum production during the period of the study, from July 26 to September 20, was about 28,000. Individual snails may have produced even more, and there is no indication as to how many miracidia had penetrated each snail; however, these reports show that the total production of cercariae in one snail may be large. It is also probable that the reproductive potential of each germinal sac is not realized because of lack of space in the host.

The germinal cells do not appear to be budded off the wall in spirorchids; but since most somatic cells contain the same chromosomal components as the germinal cells, the question of whether germinal cells are produced in the body cavity from other germinal cells or from the body wall does not seem so important as previously. The small, darkly staining cells which were noted particularly in the developing daughter sporocysts are the cells which the proponents of the idea of parthenogenesis in digenetic trematodes considered polar bodies; however, as early as 1906, Rossbach noted that these cells may be grouped in numbers greater than three around each germinal cell and that they are also found in somatic tissue. Cable (1934) considered them degenerating nuclei. Miller (1950) thought they were either pyknotic nuclei or newly formed daughter cells in which the chromosomes had not yet entered into the typical resting stage of the nucleus. Because of the variability of the appearance of these darkly staining nuclei and their presence in

somatic tissue, it appears to the writer that they may be either cells in which the chromatin has not yet differentiated or is disintegrating.

Failure to find polar bodies or any other evidence of maturation phenomena in *Spirorchis artericola* excludes the hypothesis of polymorphism for that species The chief advocate of this theory is Woodhead, who studied the germinal cycle in three species of BUCEPHALIDAE (1931) and claimed that three generations of sexually reproducing adults were present. According to La Rue (1926) the blood flukes and the bucephalids are closely related. The failure of the writer to find any indication of maturation may mean that the bucephalids are not related to the blood flukes nor perhaps even to the other TREMATODA classified as DIGENEA. Yet Woodhead claims to have found evidence for polymorphism in incomplete studies of members of the family BRACHYLAEMIDAE (1932, 1950) as well as in *Paragonimus kellikotti* (1951) and *Schistosomatium douthitti* (1952). This divergence of interpretation concerning the trematode germ cell cycle in general and *Schistosomatium douthitti* in particular shows the need for further detailed investigations by workers in various locations.

SUMMARY

The life history of *Spirorchis artericola* has been determined and its germ cell cycle traced through the larval stages in the intermediate host, *Helisoma trivolvis*. The normal primary host in Swan Lake, Iowa, is *Chrysemis picta bellii*. Adult worms and eggs containing viable miracidia were recovered from *Chrysemis picta dorsalis* four months after exposure to cercariae emerged from laboratory infected snails.

Eleven to fifteen germinal cells are located in the posterior part of the body cavity of the free-swimming miracidium. After the miracidium has penetrated the intermediate host, it becomes fixed in tissue, usually the mantle, and develops into the sac-like mother sporocyst. Germinal cells within the body cavity grow and divide mitotically to produce enough germinal cells to fill the body cavity and replace the degenerating somatic tissue of the miracidium.

At water temperatures between 24° and 28° C., young daughter sporocysts begin to form in the mother sporocyst sixty to seventy-two hours after infection. Cleavage of individual germinal cells results in two cells of unequal size. The larger or somatic cell gives rise to the soma of the daughter sporocyst. The smaller or germinal cell retains the germ plasm in its descendent germinal cells. After the third day of development the mother sporocyst elongates rapidly by flattening of its wall cells and growth at the ends. While the daughter sporocysts are still within the mother sporocysts, some of the germinal cells within the daughters divide mitotically into a somatic and a germinal cell. The somatic cells divide repeatedly, but the germ plasm remains segregated in several germinal cells. As early as eleven days after infection daughter sporocysts containing germinal cells and a few germinal balls migrate from the mother along the lymph spaces into the digestive gland of the snail. The germinal cells of the developing cercaria divide repeatedly to form many small cells, arranged in a single genital primordium in the posterior region of the cercaria. At the same time somatic cells differentiate into the various cercarial tissues and organs. Beginning the twenty-first day after infection, the mature cercariae leave the daughter sporocysts through the birth pores and enter the sur-

rounding water. If a suitable turtle is encountered, they penetrate it and develop into adult spirorchids. The cells of the genital primordium become differentiated into testes, ovary, and accessory glands. Germinal cells remain in the body cavity of the germinal sacs throughout their existence. These may slowly differentiate into the proper generation.

The theory of germinal lineage with multiplication by simple polyembryony may be applied to *Spirochis artericola.* No suggestion of maturation phenomena or polar body formation and no indication of the formation of germinal cells from the body wall were found. In the mother and daughter sporocysts germinal cells grow, divide, and develop directly into daughter sporocysts and cercariae, respectively. In the cercariae they divide to form the genital primordium.

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Explanation of Plates

Figs. 1 through 17 were drawn with the aid of a camera lucida. Figs. 18 through 31 are photomicrographs. Figures enlarged to common scales are as follows: (a) 1 through 17; (b) 18 through 21; (c) 22, 23; (d) 25 through 29, 31.

Abbreviations: a, genital anlage; d, daughter sporocyst; g, germinal cell; m, mitotic cell; s, somatic cell; v, vitelline or investing cell.

Plate I

Fig. 1. Germinal cell in miracidium.
Fig. 2. Germinal cell in daughter sporocyst from 19-day infection.
Fig. 3. Germinal cell in daughter sporocyst from snail emitting cercaria over one month.
Fig. 4. Two-cell daughter sporocyst.
Fig. 5. Two-cell cercaria.
Fig. 6. Three-cell daughter sporocyst.
Fig. 7. Three-cell cercaria.
Fig. 8. Four-cell daughter sporocyst.
Fig. 9. Five-cell cercaria from twelve day infection.
Fig. 10. Ten-cell cercaria. Whole mount pressed under coverslip.
Fig. 11. Ten-cell daughter sporocyst. Teased from mother sporocyst.
Fig. 12. Sections of fifteen-cell daughter sporocyst from seven-day mother.
Fig. 13. Sections of twenty-four cell cercaria.
Fig. 14. Twenty-nine cell daughter sporocyst teased from mother and pressed under coverslip.
Fig. 15. Germ cells from mature worm, (a) primary oocyte, (b) primary spermatocyte showing tetrads, (c) sperm.
Fig. 16. Mitotic germinal cells in mother sporocyst.
Fig. 17. Mitotic germinal cells in daughter sporocyst.

Plate II

Fig. 18. Cross section of miracidium fifteen minutes after penetration into snail foot.
Fig. 19. Longitudinal section through fifty-three hour mother sporocyst.
Fig. 20. Oblique section through sixty-hour mother sporocyst.
Fig. 21. Longitudinal section through sixty-hour mother sporocyst.
Fig. 22. Longitudinal section through portion of cercaria in daughter sporocyst. Note genital primordium and gland nuclei.
Fig. 23. Cross section of daughter sporocyst and cercaria.
Fig. 24. Longitudinal section of body of mature cercaria in sporocyst.

Plate III

Fig. 25. Longitudinal section through seventy-two hour mother sporocyst.
Fig. 26. Longitudinal section of portions of seven-day mother sporocyst.
Fig. 27. Oblique section of portion of fifteen-day mother sporocyst containing young daughters and single germinal cells.
Fig. 28. Oblique section of portions of mother sporocyst.
Fig. 29. Cross section of mother sporocyst.
Fig. 30. Longitudinal and oblique sections of daughter sporocysts on digestive gland of snail. Twelve day infection.
Fig. 31. Portion of daughter sporocyst containing sections of cercarial tail, furcae, and mass of germinal cells. Nineteen-day infection.

PLATE I

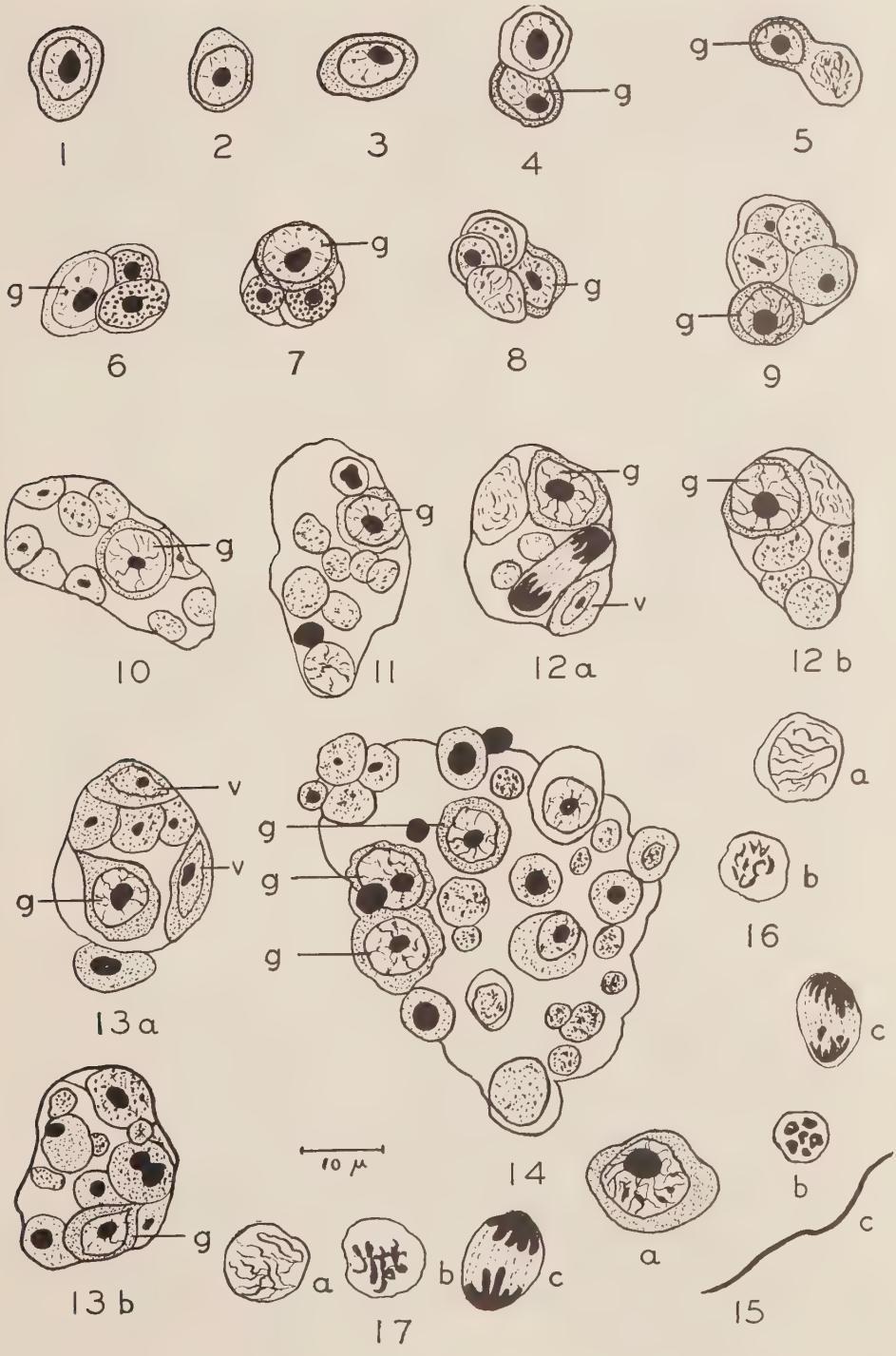
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PLATE II

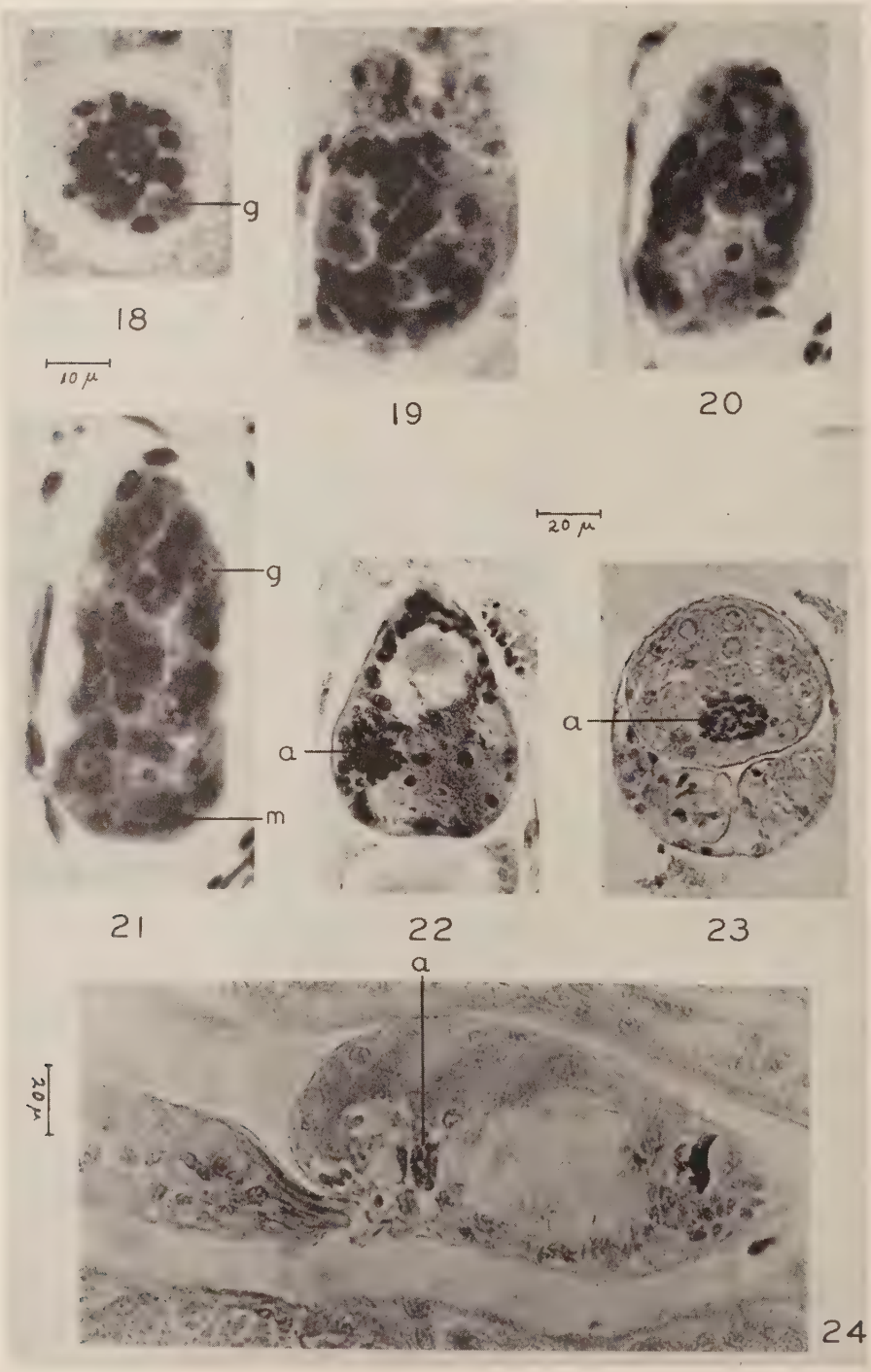
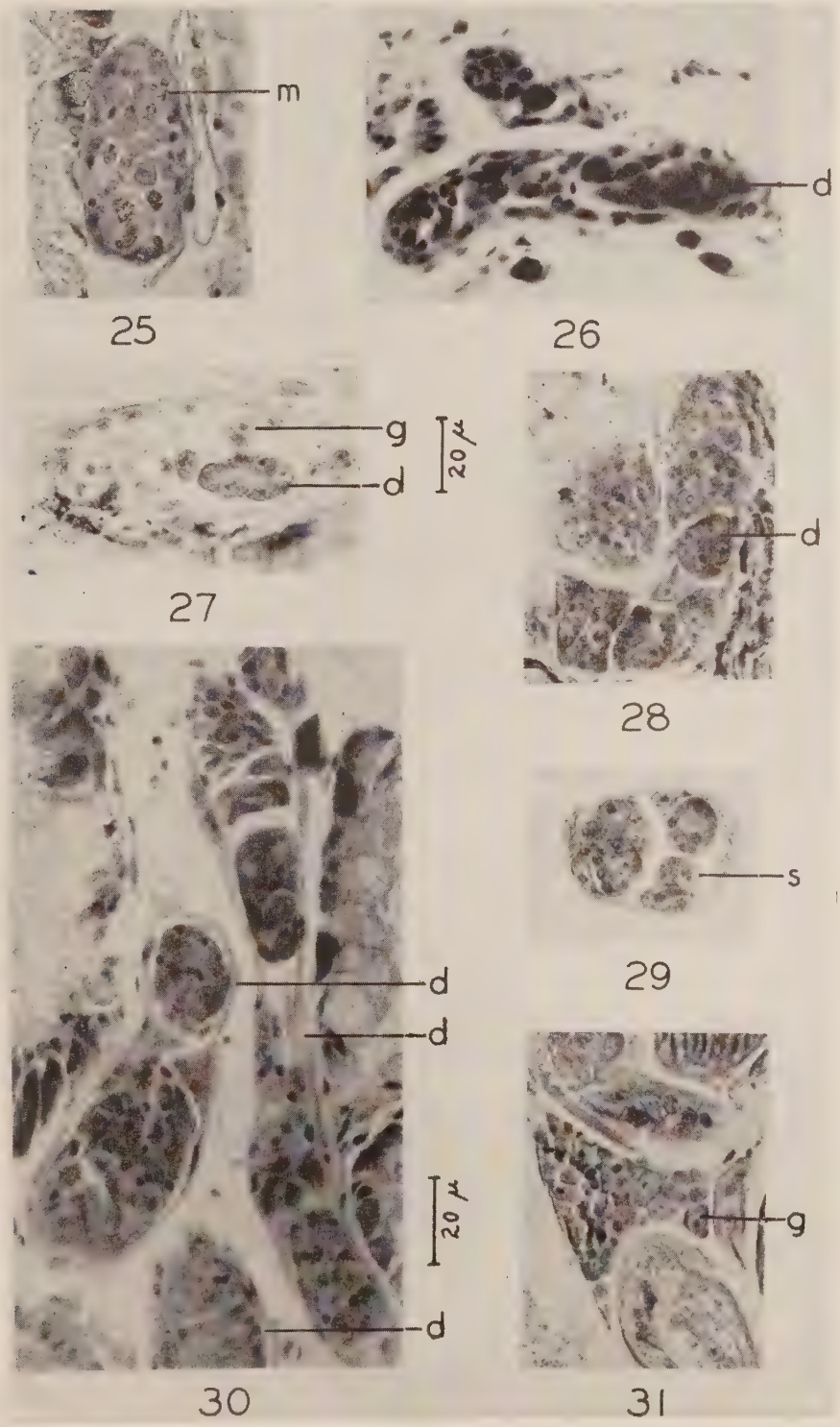


PLATE III



A STUDY OF THE PHYSIOLOGY OF BACTERIA WHICH SUPPORT THE GROWTH OF *ENDAMOEBA HISTOLYTICA* IN VITRO

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The cultivation of *Endamoeba histolytica* with *Trypanosoma cruzi* (Phillips, 1950; Phillips and Rees, 1950) represents the first time this amoeba has been grown successfully in the complete absence of bacteria or bacterial products. As a vehicle for testing amoebicidal agents, the culture has proved to be practicable and reliable (Bradner and Rawson, 1951; Nakamura and Anderson, 1951). Since the trypanosomes die off naturally under the environmental conditions imposed by the Phillips culture, it seems apparent that this culture would be especially suited to experiments envolving attempts to grow the amoeba in association with different organisms. The literature reveals several methods used in the past to test for the amoebiontic (amoeba-supporting) ability of different bacteria. Dobell and Laidlaw (1926) used acid or acriflavin-washed cysts of *E. histolytica* which were placed in tubes of various media and bacteria thus establishing the amoebae with a new flora. Cleveland and Sanders (1930) used the sterile liver abscesses of amoeba infected cats as a trophozoite source in a similar experiment with single species of bacteria. Chinn, Jacobs, Reardon and Rees (1942) made the most comprehensive search for amoebionts to date. Using bacteria-free cysts for amoeba inoculum twenty-six species of bacteria (41 strains) were tested with regard to their ability to cause excystation and growth of *E. histolytica* in vitro. A heterogenous group of 14 species of bacteria were found capable of doing so. Jacobs (1950) made a compilation of bacterial amoebionts from several sources. Also, he added four more to the list as a result of his experiments using amoebae growing with *Streptococcus hemolyticus* in which the latter was eliminated with penicillin and replaced by other types.

Although many organisms have been found capable of sustaining the amoeba in vitro, little information concerning the physiological properties of such organisms has been published. It is generally agreed that since it would be difficult to study the physiology of *E. histolytica* when other organisms are present, a study of the amoebionts might possibly reveal similarities amongst them which would explain their function in supporting growth of the protozoan. It also seems apparent from the literature that an improved, standardized method of isolating the amoeba for use in floral tests would be helpful.

Thus the research described in this paper has three purposes: first, to evaluate the Phillips' method as a source of bacteria-free amoebae in cultural studies; second, to study the major physiological activities of bacterial amoebionts isolated in this laboratory; and third, to indicate similarities in the metabolism of supporting organisms which might be of aid in the search for growth factors for the amoeba.

Received for publication, October 30, 1952.

The author acknowledges with thanks the advice and criticism of Professors S. J. Thomas, B. W. Parker, and T. H. Grainger of Lehigh University, and of Dr. C. W. Rees and Mr. B. P. Phillips of The National Institutes of Health.

MATERIALS AND METHODS

Three strains of *Endamoeba histolytica* (F-22, 200, and 103) were obtained from the National Institutes of Health in Phillips' culture. Also received from this source was the Culbertson strain of *Trypanosoma cruzi* growing in a blood agar medium. These cultures were maintained by procedures similar to those employed at the National Institutes of Health.

With the exception of *Clostrium perfringens, organism "t",* and *"streptobacillus"* (supplied by N. I. H.) all bacteria were secured from the Lehigh University stock collection. Numbers, when given, are designations assigned by the University for convenience only. All aerobes were supplied from stock either in thioglycollate broth or agar slants. From these, plates were streaked and a single colony picked into thioglycollate broth which, after 24–48 hours incubation, was used as the source of bacteria for the amoebiontic test. No attempt was made to characterize the organisms at this point so long as the mass growth and morphology were within normal limits. Anaerobes were supplied in thioglycollate broth from which aerobic agar plates were streaked as a check for possible contamination by aerobes. Although no specific tests were made for cross-contamination of anaerobes, the consistency with which characteristic growth was repeated in thioglycollate broth, the homogeneity of stained slides, and the results of subsequent physiology tests (in comparison with Bergey's descriptions) attested to their probable purity. Following a test with amoebae the bacteria were transferred by loop from the last tube to agar slants or thioglycollate broth (depending on O_2 requirements), incubated at 37° C. for 24 hours, and retained at 20° C. until the physiology could be checked.

As reported by the writer in a recent publication (1952) considerable experimentation went into the selection of a medium for the amoebiontic test. The "C & C" medium (Cleveland's liver-infusion agar) was finally chosen as it seemed to be slightly more consistent in results as well as providing a richer medium for bacterial growth. Preparation was as follows: *Difco Endamoeba Medium* was rehydrated and tubed in 5 ml. amounts, then autoclaved and chilled to harden in a steep slant. Each slant was covered with 7.5 ml. of buffered 1 : 10 serum-saline solution. About 30–40 mg. of sterile Bacto rice powder were added to each tube just prior to inoculation.

PROCEDURE FOR SELECTING AMOEBIONTIC BACTERIA

Three tubes of C & C medium were each inoculated with 0.1 ml. from a 24–48 hour thioglycollate broth culture showing abundant growth of the bacteria to be tested. These tubes were then incubated 24 hours at 37° C. after which each was inoculated with 0.5 ml. from a Phillips' amoebae tube—the first with *E. histolytica* str. *F-22,* the second with str. *200,* and the third with str. *103.* After the tubes had been incubated again for 4 days at 37° C. several drops were taken from each (at the rice starch-overlay interface) and examined under both low and high dry powers of a microscope. If a tube was negative (no amoebae present in sample) it was held for another three days and examined again. If still negative, it was discarded. All positive tubes (any amoebae present, condition notwithstanding) were transplanted to fresh, warmed C & C medium using 0.5 ml. of the mixed sediment from the bottom of the test culture tube. Serial transplants were made at least twice to

be assured that the amoebae in the original test inoculum were diluted out so as to ascertain whether or not growth and multiplication were taking place.

Any tubes of a dubious nature (e.g. failure of bacteria to grow; appearance of a contaminant) were discarded and the test was repeated. In many cases one or more duplicate tests were run on each type of bacteria and all "positives" were repeated several times as well as being submitted to a longevity test of serial subculturing for a period of five weeks.

Amoebiontic tests were run on 4–6 species of bacteria at a time. With each such group a tube containing organism "t" was inoculated with amoebae as a control on the viability of the protozoan. There was no instance where a good growth of organism "t" failed to support the 103 and 200 strains of *E. histolytica*, though variations in population densities occurred from time to time. On the other hand, the F-22 strain failed to grow satisfactorily at any time in C & C medium with organism "t".

BACTERIAL PHYSIOLOGY TESTS

Bacteria from tubes set aside for testing were plated out on nutrient agar to establish purity of the culture. Each type was then grown 24 hours in thioglycollate broth which in turn was used as the source of inoculum for the physiology tests. Two principles governed the selection of tests for the active bacteria. These were, first, tests which would give a good general outline of the metabolic capabilities of each species and second, tests where standardized media could be used as much as possible in order to make any comparisons valid. The 21 carbohydrates were made up in 0.5% concentration in Trypticase Agar Base (Baltimore Biological Laboratories #151). This medium served both aerobes and anaerobes. Other tests were made using special media devised for *Clostridia* by Reed and Orr (in Kolmer and Boerner, 1945) in addition to standard prepared media (Difco Manual). Before use all media were incubated for at least 24 hours to ascertain sterility. Control tests were run when necessary on bacterial species which were known to give typical reactions. If controls were unsatisfactory, the entire test was repeated. A blank tube of each type of medium was also submitted to test procedures.

RESULTS

The eight bacteria listed below whose major physiological responses are reported were chosen on the basis of being able to stimulate the growth and reproduction of at least one strain of *E. histolytica* as evidenced by the presence of motile trophozoites on microscopic slide examination following: 1. repeated successful transfers from Phillips' culture to bacterial culture, and, 2. an extended series of serial subcultures from one transfer.

Organism	*Abbreviation used in table*
Clostridium tetani	Cl. tet.
Clostridium botulinum	Cl. bot.
Clostridium pasterianium 79L	Cl. past.
Clostridium perfringens	Cl. perf.
Bacillus circulans	B. circ.
Micrococcus pyogenes var. *aureus* 9L	S. aureus
Micrococcus pyogenes var. *albus* 43L	S. albus
Paracolobactrum coliforme Pv 33R	Pv 33R

All the above organisms except *C. perfringens* and *S. aureus* are not previously reported in the literature as possessing the amoeba-supporting ability.

The following bacteria gave some slight support to the amoeba as evidenced by the presence of typical motile trophozoites on at least one occasion:

Alcaligens fecalis	*Clostridium histolyticum* 98L
B. anthracis	*Proteus vulgaris* (sewage isolate)
B. brevis	*Salmonella enteriditis*
B. megatherium	*S. pullorum*
B. subtilis var. *globigii*	*Shigella dysenteriae* (Shiga)
B. subtilis 69L	*S. dysenteriae* (Sonne)
B. thermophilus	*M. Pyogenes* var. *aureus* 68L

With 43 other types of bacteria no motile amoebae were seen, although non-motile and degenerate forms could sometimes be found after one, or at most, two subcultures.

Recording of physiology tests:

–	no reaction
a	trace of acidity
a	slightly acid
A	Acid
g	slight gas formation
Gas	Gas
+	reaction positive

Incubation times: carbohydrates 7 days; other tests, as indicated (in days) by a numeral in parenthesis following the reaction title.

DISCUSSION

In separating amoebionts from non-supporting organisms for comparison, as many factors as possible were kept identical or equivalent so that in the end the bacteria were the only gross variable in the experiment. In adhering to such conmedium as a few instances of feeble reproduction were obtained in some tests with ditions it is obvious that many potential amoeba-supporting bacteria have not been so credited. Hence, a comparison of the physiologies of so-called non-supporting organisms would not be valid. Much of the earlier literature on bacterial amoebionts seems to imply that the supporting ability is strictly a species characteristic. The results here obtained with several micrococci seem to indicate a wide variance of ability between strains of the same species of bacteria. Likewise, an analogous difference in ability to utilize bacteria on the part of different strains of amoebae was demonstrated. The F-22 strain of *E. histolytica* failed to reproduce effectively with any of the bacteria. This result may have been a function of the egg media. Strains 200 and 103 closely paralleled each other in their bacterial preferences though 200 seemed somewhat hardier.

It is felt that the feasibility of the use of the Phillips' culture as a source of bacteria-free amoebae for cultural studies has been firmly established. The methods using washed cysts as an amoeba source always leave the question that failure to ex-cyst rather than lack of supporting ability on the part of the associate being tested

may be the reason for negative results. The use of trophozoites grown with penicillin-sensitive bacteria—the latter being eliminated with the antibiotic and replaced with other organisms—has two particular drawbacks: other penicillin-sensitive bacteria cannot be tested and penicillinase producers cannot be tested. The use of sterile cat-liver abscesses or of micromanipulator isolation as bacteria free trophozoite sources present complexities of operation which render them impractical as methods for mass testing. All these difficulties are obviated with the Phillips' culture.

From the physiological tests performed it can be seen that none of the organisms fermented adonitol or dulcitol and that none of them produced either indol or acetylmethylcarbinol. These results cannot be regarded as significant until they can be compared with the examinations of a great many more amoebiontic bacteria. However, it is felt that comparisons of this type may eventually lead to the discovery of the metabolic properties necessary for the support of enteric amoebae.

Besides serving as a point of departure in the search for growth factors for *E. histolytica,* this work reveals that the supporting ability is a fairly stable characteristic and might be added to the long list of tests for differentiating bacterial types —especially strains of the same species. The possibility exists that in a like manner different species of *Endamoeba* and even strains of *E. histolytica* could be identified by their "spectra" of amoebiontic bacteria. The latter hypothesis, if eventually proven by further work, might be of importance to the pathologist in indicating the most effective therapy for amoebiasis.

Summary

(1.) Sixty-five bacterial types were tested for their ability to support the growth of three strains of *Endamoeba histolytica.* (2.) This ability was most pronounced in eight bacterial types six of which have not previously been reported in the literature. (3.) Some of the physiological characteristics of these bacteria are reported

TABLE 1.

		Cl. tet.	Cl. bot.	Cl. past.	Cl. perf.	B. circ.	S. aureus	S. albus	Pv 33R
Gelatin liq.	(5)	slow	+	–	+	+	–	–	–
Indol	(1)	–	–	–	–	–	–	–	–
Methyl red	(5)	–	–	+	–	+	–	+	+
V. P.	(1)	–	–	–	–	–	–	–	–
H_2S form.	(1)	–	–	–	+	–	slight	slight	+
Urease	(2)	–	–	–	–	+	+	+	–
NO_2 from NO_3	(1)	–	–	–	+	–	+	–	+
Litmus milk	(5)	–	A. black	–	A. coag.	a	A. coag.	A. coag.	A. to alk.
Citrate util.	(5)	–	–	–	–	+	+	+	–
Fermentations :									
Arabinose		–	–	–	–	–	a	–	a
Rhamnose		a	–	–	–	–	–	–	–
Xylose		a	a	–	–	–	a	–	Ag
Glucose		–	AG	AG	AG	A	a	A	Ag
Fructose		–	a	AG	AG	A	a	A	A
Galactose		–	a	AG	AG	–	a	A	Ag
Mannose		–	–	Ag	AG	A	a	A	A
Lactose		–	–	–	AG	–	–	a	Ag
Sucrose		–	–	AG	AG	a	a	A	AG
Starch		–	AG	–	AG	A	–	a	A
Inulin		–	–	AG	–	–	–	–	–
Dextrin		–	a	–	AG	A	–	–	Ag
Glycerol		–	–	–	A	–	a	a	a
Adonitol		–	–	–	–	–	–	–	–
Mannitol		–	–	–	–	–	a	a	a
Sorbitol		–	Ag	–	–	–	a	–	–
Dulcitol		–	–	–	–	–	–	–	–
Salicin		–	AG	–	–	–	–	a	a
Maltose		–	AG	AG	AG	A	a	A	Ag
Trehalose		–	Ag	–	AG	A	a	A	A
Raffinose		–	–	–	AG	–	–	–	A

and some similarities amongst them are noted. (4.) The Phillips' culture is deemed a suitable source of bacteria free trophozoites for cultural experiments and in some respects superior to sources reported in the past. (5.) Possible applications of information gained from studies of this type are discussed.

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A NEW COPEPOD PARASITE FROM THE GRUNION

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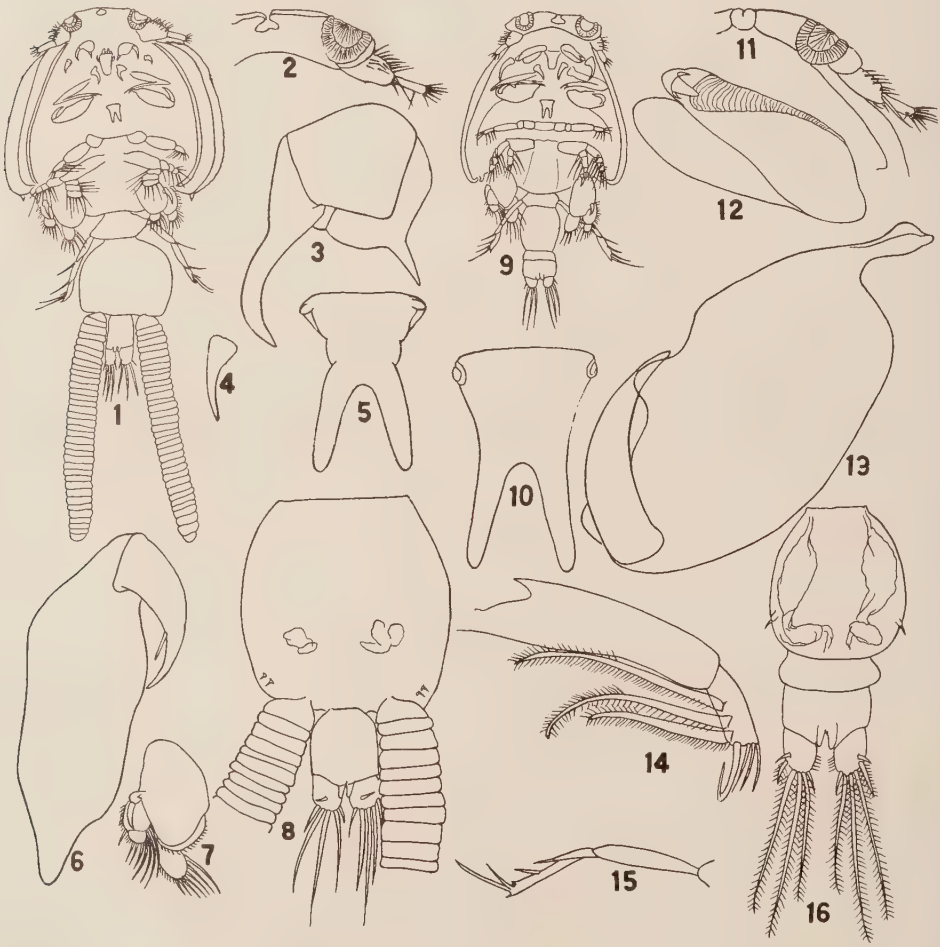
Mr. Andrew C. Olson, Jr., Assistant Professor of Zoology, San Diego State College, kindly sent me about forty specimens that he collected at Mission Beach and along the shore of San Diego Bay in California. In one collection he obtained 38 copepods from 42 fish. The host in all cases was the grunion, *Leuresthes tenuis* (Ayres).

Caligus olsoni n. sp.

Female: Carapace about as long as remainder of body; somewhat longer than wide; narrowed anteriorly. Frontal plates wide; lunules large, flattened anteriorly. Posterior sinuses



Figs. 1–16. 1, female; 2, first antenna; 3, second antenna; 4, first maxilla; 5, furca; 6, second maxilliped; 7, third leg; 8, genital segment, abdomen, caudad rami, and egg strings; 9, male; 10, furca; 11, first antenna; 12, second antenna; 13, second maxilliped; 14, tip of first leg; 15, fourth leg; 16, genital segment, abdomen, and caudal rami.

Received for publication, December 4, 1952.

narrow; median lobe projects a little behind the lateral lobes, somewhat more than half the entire width, lateral lobes rather narrow with a narrow median portion that is rounded at tip, as is the wider lateral part. Carapace generally smooth and not broken up by grooves.

Free thoracic segment short and narrow, less than half as wide as genital segment. Genital segment about as wide as long, with curved lateral margins; with a pair of short setae near the posterior end on each side. Abdomen 1-segmented, four-fifths as long as genital segment and about a third as wide. Anal papillae half as long as abdomen, with a short dorsal, three long and two short setae. Egg strings shorter than body, with about 33 eggs.

First antenna prominent but extending very little laterally. Second antenna with a stout basal segment that bears a strong posterior spine; terminal hook long, curved and sharp. First maxilla short, slightly curved, pointed, with base slightly swollen. Second maxilla with a wide base and curved terminal hook. First maxilliped of the usual form, terminal claws short. Second maxilliped rather slender, terminal hook with a seta on its inner margin. Furca with straight branches about the same length as the base, which is wide and straight across front with two small lateral lobes. First leg uniramous, terminal segment with three long, one medium, and three short setae; basal segment with a short anterior seta. Second leg biramous with 3-segmented rami; spines on first and second segments of exopod very short. Third leg with comparatively narrow basal segment, lateral hook small; exopod 2-segmented, endopod 1-segmented. Fourth legs slender, weak, 3-segmented, setae short.

Length of body, 3.8 mm.; carapace length, 1.95 mm., width, 2.0 mm.; genital segment length, 0.9 mm., width, 1.0 mm.; abdomen length, 0.75 mm. Type. U. S. Nat. Mus. No. 93733.

Male: Similar to female, but the second antenna is corrugated on its ventro-anterior surface, has a rather slender basal segment, and a much shorter terminal hook than the female. Also, the abdomen is 2-segmented, the basal segment being half as long as the second segment. The genital segment has a single seta on either side near its posterior end. The furca is a little narrower in front, and has smaller lateral lobes.

Length of body, 3.8 mm.; carapace length, 1.95 mm., width, 2.0 mm.; genital segment length, length, 0.6 mm., width, 0.5 mm.; abdomen length, 0.5 mm. Type. U. S. Nat. Mus. No. 93734.

This species somewhat resembles *Caligus schistonyx* Wilson (1905) but the second antenna of the male lacks terminal setae and instead has two small lateral spines; the female first leg does not have bifid terminal setae; the male carapace is relatively narrower than that of the female; the female genital segment does not have posterior lateral lobes; and the furca has a different base and its arms are straight. The species is named for the man who discovered it.

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A NEW SPECIES OF *SARCOPTES* (ACARINA: SARCOPTIDAE) FROM THE CAVE BAT

Robert A. Hedeen*

A new species of *Sarcoptes* was collected from the cave bat, *Myotis velifer* (Allen), in central Texas during the period 1951–1952. One specimen was taken from the above named host at Camp Bullis, Bexar County, Texas, in 1951. Three additional specimens were collected near Austin, Travis County, Texas, in 1952. The parasites were found near the oral region of the bats, always in an anomalous tube-like structure which protruded conspicuously. Infested bats always exhibited a face extremely swollen and containing numerous pus pockets. Efforts to find additional mites in the deeper facial tissues were unsuccessful. The "tube" was quite obvious and could be readily removed with fine-pointed scissors. All such structures examined yielded one mite and several eggs. It is the writer's opinion that the mites normally infest the deeper facial regions but come to the peripheral region to oviposit and that the tube-like structure is a protective reaction of the host to the parasite's activities. At Camp Bullis one of 20 *Myotis velifer* was found to be infected and three of 12 from Austin were found to harbor the parasite. Two hundred Mexican free-tailed bats, *Tadarida mexicana* (Saussure), from a cave also at Camp Bullis were examined and no mites of this type found.

Sarcoptes myotis, new species

Adult female: Body subglobose, about 385 microns in length and about 270 microns in width. Convex dorsally without an armature of scales and spines. Setae few, very small and scattered over the dorsal surface. Two large subterminal spines, one on each side of the anal opening, projecting posteriorly. Integument arranged in fine parallel ridges over the surface of the body, except where they curve around the anal opening. Plastron a small, faintly appearing, rectangular area located on the dorso-anterior part of the body. A minute seta on each side of the plastron. Plastron devoid of integumental ridges. Eyes lacking. Suture between notogaster and notothorax lacking. Anal opening terminal, folding back dorsally. Copulatory papilla a flat, scale-like structure located just anterior to the anal opening.

Capitulum: Consists of chelicerae, pedipalps, and basis capituli or rostrum, the widest point being about 90 microns. The mouthparts are enclosed in a capsule formed by anterior growths of the integument; the dorsal of which is the epistome and bears two pairs of setae. The posterior pair of setae are small and do not project beyond the lateral edges of the capitulum. The anterior pair are large and project beyond the lateral edges of the capitulum. Palpi dorsolateral in position and three segmented, the most apical segment not bifurcated. The chitinized bases of the palpi are continuous around the rostrum, giving support to it. Chelicerae a little longer than the palpi with both movable and non-movable fingers strongly toothed.

Anterior legs: Anterior two pair inserted in an anterolateral position, easily seen from the dorsal aspect, and identical in structure. Each leg consists of five segments which are somewhat telescoped and delimited by rings of chitin. The most apical segment bears three claws and the ambulacrum which is less than half the length of the leg. The peduncle of the ambulacrum is unsegmented and is constricted just basally to the terminal "bell." The bell is only a little larger in diameter than the peduncle. The epimere of each of the first pair of legs projects posteriorly until it meets the other just posterior to the basis capituli. From this point they project posteriorly as the sternum, each epimere retaining its identity. The sternum extends posteriorly to a point just anterior to the ends of the epimeres from the second pair of legs.

Posterior legs: The two hind pairs are identical in structure and due to a more medial in-

Received for publication, November 24, 1952.

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sertion may not be seen from the dorsal view. They also differ from the anterior legs in that they consist of only four, chitin-delimited, telescoped segments and no suckers are present. A long seta on each leg, subequal to the length of the leg itself, apparently represents the missing sucker. The epimeres of the legs project inward for a short distance but do not unite.

Type host: The cave bat, *Myotis velifer* (Allen)

Type habitat: Oral region

Type locality: Texas, Travis County

Type material: Holotype deposited in the U. S. National Museum, number 2109. One paratype deposited in the University of Texas entomological collection, and two paratypes retained in the author's collection.

Sarcoptes myotis, new species, increases the number of species in this genus to three. Prior to 1950 only one species, *S. scabei,* with several varieties, was known. Boyd and Bernstein (1950) described a second, *S. lasionycteris,* from the bat *Lasionycteris noctivagans* (LeConte). *S. myotis,* new species, differs from *S. scabei* as described by Friedman (1942) in the following ways: absence of armature of scales, cones, and spines on the dorsal surface; decreased length of long seta on posterior two pair of legs; first epimeres not uniting completely in the formation of the sternum; and in a number of minor ways such as smaller size and reduction in body setae. *S. myotis* differs from *S. lasionycteris* in the following major ways: body subglobose; apical segment of palpi not bifurcate; three claws on anterior tarsi; and in a number of minor differences.

SUMMARY

Described as a new species is *Sarcoptes myotis* from the cave bat, *Myotis velifer* (Allen), in Travis County, Texas. Holotype female and three female paratypes were used in preparing the description. The mites were found in peculiar tubes of tissue on the face of the parasitized host. Specimens have been collected from two localities in the region of central Texas.

ACKNOWLEDGMENT

The writer wishes to express his appreciation to 1st Lt. Ray Applewhite, MSC, U.S.A.R., who aided him in collecting the bats at Camp Bullis, and who was most helpful in other aspects.

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COLLASTOMA PACIFICA SP. NOV., A RHABDOCOEL TURBELLARIAN FROM THE GUT OF *DENDROSTOMA PYROIDES* CHAMBERLIN

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INTRODUCTION

Examination of two specimens of the sipunculid worm *Dendrostoma pyroides* Chamberlin collected in 1949 at Hopkins Marine Station, Pacific Grove, California, disclosed the presence in one of them of five individuals of an undescribed species of *Collastoma,* for which I propose the name *Collastoma pacifica.* The rhabdocoels inhabit the anterior half of the gut of the host. In 1952, Mr. Lee Douglas examined three specimens of *D. pyroides* taken at Carmel Point, California, and found two of them to be infected by five worms each. I am indebted to Mr. Douglas for making this additional material and notes on living specimens available to me for study.

All known species of *Collastoma* occur in the gut of sipunculids. The genus was established by Dörler (1900) for *C. monorchis,* from *Phascolosoma vulgare* Diesing collected at Roscoff. Wahl (1906) made brief reference to a form which he called *C. minuta,* taken from *Physcosoma granulatum* Leuckart at the Bay of Naples. In a later paper (1910b) he gave a full description of this species, the specific name of which he emended to *minutum.* A third species, *C. eremitae,* occurring in *Phascolosoma eremitae* M. Sars in the Gulf of Kola, was described by Beklemishev (1916)

Wahl (1910a) established the family UMAGILLIDAE and two subfamilies, COLLASTOMINAE and UMAGILLINAE. The former includes, at present, only the genus *Collastoma.* A third subfamily, BICLADINAE, was proposed by Stunkard and Corliss (1950). The family UMAGILLIDAE has recently been reviewed by Stunkard and Corliss (1951).

METHODS

The rhabdocoels were removed from the gut of the host in sea water. Some of them were flattened slightly by pressure from a cover glass during fixation in Bouin's or Brasil's fluids, and used for preparation of whole mounts stained in borax carmine or alum hematoxylin. Specimens used for transverse or longitudinal sections were fixed in Brasil's fluid, embedded in paraffin, and cut serially at 10 microns. The sections were stained in iron hematoxylin and fast green.

DESCRIPTION

The living animals of *Collastoma pacifica* have a light pinkish color, and are dorsoventrally flattened. Viewed from the dorsal or ventral aspect they are more or less oval. In life, the largest specimens measured approximately 2 mm. in length when extended.

The epithelium covering the dorsal surface and the anterior, posterior, and lateral areas of the ventral surface is ciliated, but a considerable portion of the epithe-

Received for publication, November 3, 1952.

lium of the ventral surface around the midline is unciliated. The epithelial cells in the mid-dorsal region are columnar and approximately 15 microns in height, with cilia about 5 microns long. The epithelium of the mid-ventral surface is composed of cells which are more nearly cuboidal, being as a rule only slightly higher than wide; the largest of these cells are about 15 microns in height. In the midline, the unciliated area of the ventral surface extends from the level of the posterior edge of the pharynx to a point about half way between the seminal bursa and the genital pore. In the posterior region of the ventral surface, both the ciliated and unciliated cells are relatively short.

Beneath the epithelium there is a thin sheet of muscles. The outermost layer of this muscular sheet is composed of circular fibers, and the innermost layer consists of longitudinal fibers. There are oblique fibers between the layers of circular and longitudinal fibers. Dorsoventral muscle fibers are scattered through the parenchyma.

The brain, composed of two ganglionic masses connected by a commissure, lies a short distance anterior to the pharynx. In sectioned material it is possible to trace for a short distance three major nerve trunks which extend anteroventrally, posteroventrally, and posterodorsally from each ganglionic mass.

The mouth is a small opening on the ventral surface near the end of the first one-fifth of the body. The pharynx is strongly muscular and is similar in form to that of other umagillids. The intestine extends posteriorly as far as the end of the fourth one-fifth of the body. The cells of the intestine are large and irregular, with abundant inclusions.

The single testis is elongated and is ventral to the intestine. Its length is about one-third the length of the body. The anterior end of the testis lies beneath the pharynx. Posteriorly, it becomes narrowed as it passes into the sperm duct. The seminal vesicle is ovoid and enveloped by a sheath of muscle fibers. In my material the seminal vesicle is full of sperm. The portion of the sperm duct which leads from the seminal vesicle to the genital antrum is slender and chitinized; this portion is usually referred to as the penis. There is a small hemispherical protuberance, possibly glandular in nature, on either side of the origin of the chitinized portion of sperm duct.

The paired vitellaria lie on either side of the testis and extend anteriorly to the level of the posterior edge of the pharynx. In individuals which have been compressed during fixation, the vitellaria may extend anteriorly as far as the mouth. The vitellaria in contracted individuals show undulations and alternating dilations and constrictions.

The paired ovaries are posterior to the vitellaria. Anteromedially, each ovary becomes narrowed and joins the posterior end of the vitellarium on the same side to form a short ovovitelline duct which enters a duct originating from the anteroventral side of the seminal bursa. Dörler named this duct the ductus communis. It enters the genital antrum posterior to the entrance of the sperm duct. The seminal bursa is a large vesicle with a very thin tunic, and contains abundant sperm and a few yolk granules. Dörler described in *C. monorchis* a blind sac dorsal to the seminal bursa and communicating with the latter. A similar structure was found by Wahl in *C. minutum* and termed by him the "Nebenblase." There is a structure dorsal to the seminal bursa of *C. pacifica* which is probably homologous with the blind sac of

Dörler and the Nebenblase of Wahl, but I have not been able to establish a connection between it and the seminal bursa. In *C. pacifica* this accessory vesicle is thin-walled, alveolar, and contains masses which have siderophilic portions suggestive of deteriorating nuclei. The vagina originates on the posteroventral side of the seminal bursa. Its posterior portion is dilated and communicates with the genital antrum posterior to the opening of the ductus communis. The uterus is a blind sac extending anteriorly from the genital antrum and lies beneath the ducts which open into the genital antrum. In individuals which are carrying an egg capsule, the uterus may extend forward as far as the level of the ovovitelline ducts. The egg capsule is translucent and yellow-brown in color, and contains several eggs and abundant yolk. It is prolonged posteriorly into a stalk which branches into two long filaments. The stalk of the egg capsule passes through the genital antrum and the filaments are coiled in the dilated posterior portion of the vagina. A pair of glands, considered by Dörler to be shell glands, open into the posterior portion of the ductus communis. Behind each of these glands there is a group of unicellular glands which evidently discharge their secretion into the genital antrum. Glands of this character in umagillids are usually regarded as cement glands, on the assumption that their secretion assists in attachment of the egg capsule to the substrate. The common genital pore opens into a depression on the ventral surface close to the posterior end of the body.

The type specimen, a whole mount fixed in Brasil's fluid and stained in borax carmine, has been deposited in the United States National Museum (U.S.N.M. No. 24697). It was taken by Mr. Lee Douglas from a specimen of *Dendrostoma pyroides* collected at Carmel Point, California, June 24, 1952.

TAXONOMY OF THE GENUS *Collastoma*

Of the four known species of *Collastoma, C. eremitae* Beklemishev is unique in having vitellaria in mature individuals divided into four to six branches. The other three species are more difficult to differentiate. According to Dörler, that portion of the sperm duct in *C. monorchis* which extends from the seminal vesicle (Dörler referred to the latter structure as a false seminal vesicle) is muscular. In *C. minutum* Wahl and *C. pacifica,* on the other hand, it is a slender chitinized tubule. However, *C. monorchis* and *C. pacifica* are similar because the ovovitelline ducts join the ductus communis near the point of origin of the latter, whereas in *C. minutum,* according to Wahl, the ovovitelline ducts enter the seminal bursa itself, anterior to the origin of the ductus communis. Judging by the figure given in Wahl's earlier paper (1906), the stalk of the egg capsule in *C. minutum* is short in comparison to the stalk in *C. monorchis* and *C. pacifica.*

It is possible that the absence of ciliation over a considerable portion of the ventral surface of *C. pacifica* may be a distinctive characteristic of this species. An absence of ciliation on the ventral surface has not been noted in any of the other members of the genus.

SUMMARY

A new species of *Collastoma, C. pacifica,* is described from the gut of the sipunculid *Dendrostoma pyroides* Chamberlin collected at two stations on the coast of central California. The relationships of the four known species of *Collastoma* are discussed.

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Explanation of Plate

Collastoma pacifica sp. nov.

Figures 1 and 3 were prepared with the aid of a camera lucida.

Fig. 1. Entire worm, ventral view.

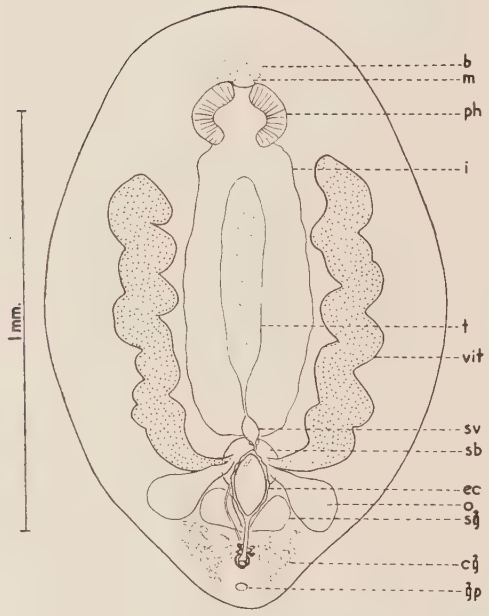
Fig. 2. Reproductive system, ventral view; semidiagrammatic; "cement glands" and "shell glands" omitted.

Fig. 3. Outline of egg capsule.

Fig. 4. Median sagittal section; semidiagrammatic.

Abbreviations: av, accessory vesicle dorsal to seminal bursa; b, brain; cg, "cement glands"; dc, ductus communis; ec, egg capsule; ga, genital antrum; gp, genital pore; i, intestine; m, mouth; o, ovary; ovd, ovovitelline duct; p, chitinized portion of sperm duct ("penis"); ph, pharynx; sb, seminal bursa; sg, "shell glands"; sv, seminal vesicle; t, testis; u, uterus; v, vagina; v^1, dilated posterior portion of vagina; vit, vitellarium.

PLATE I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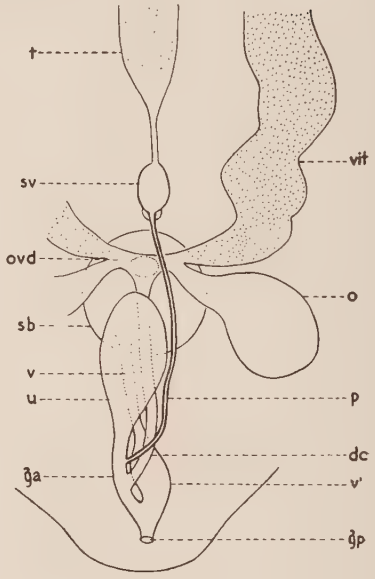
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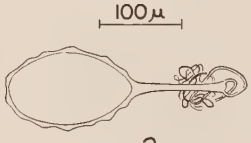
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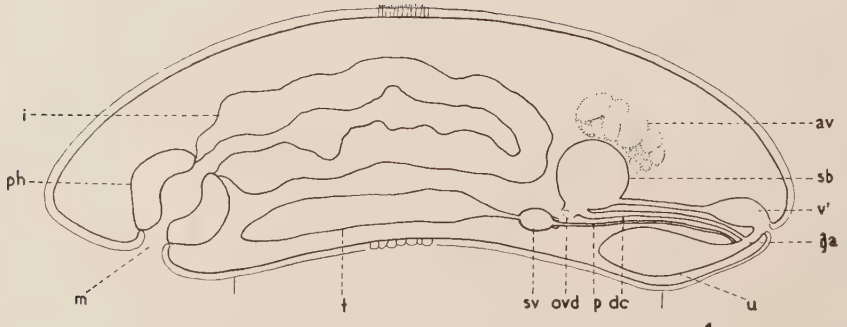
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XIPHIDIOTREMA LOCKERAE, GEN. ET SP. NOV. (TREMATODA: TROGLOTREMATIDAE) FROM SHREWS IN THE NORTHWESTERN UNITED STATES

CLYDE M. SENGER[1]
Department of Biological Sciences, Purdue University

In a survey made in the northwestern United States by Betty Locker, Kenneth Neiland, and the writer, it was found that shrews serve as hosts for a number of helminth parasites. One of these is a minute digenetic trematode which may be assigned to the family TROGLOTREMATIDAE, Odhner, although it is unique in possessing an oral sucker with a stylet plainly visible in stained adult specimens. This character and others make it impossible to assign this form to any of the existing genera of the family. Hence the author proposes the new genus *Xiphidiotrema* with *Xiphidiotrema lockerae* n. sp. as type species.

This fluke was found in four of five shrews trapped on Larch Mountain, Oregon, and in one from northwestern Montana. Some seventy other shrews from surrounding areas apparently did not harbor this form.

All of the specimens were fixed with formalin-alcohol-acetic acid solution without flattening; whole mounts were stained with either Semichon's carmine or Ehrlich's hematoxylin and serial sections with Galigher's hematoxylin and eosin.

Xiphidiotrema lockerae, n. g., n. sp. (Figs. 1–3)

(Measurements in millimeters)

Specific diagnosis: Body pyriform, rounded anteriorly and pointed posteriorly, 0.20–0.32 long and 0.10–0.17 wide. Cuticula spinose overall. Oral sucker subterminal, 0.047–0.072 in diameter, with stylet, 0.010 long and 0.003 wide, imbedded in dorsal wall. Prepharynx absent, pharynx, 0.014–0.023 in diameter. Esophagus short, intestinal ceca long, passing between testes and almost reaching posterior end of body. Ventral sucker slightly anterior to midlevel of body, 0.027–0.045 in diameter. Testes equal, opposite, in anterior half of body, and measuring 0.045–0.081 long by 0.032–0.063 wide. Genital pore median, just posterior to acetabulum. Cirrus sac posterior and to left of pore, containing seminal vesicle, prostatic cells, and a short cirrus. Ovary just posterior to ventral sucker, slightly to right of midline, and measuring 0.018–0.022. Seminal receptacle and Laurer's canal not apparent. Vitelline follicles large, scattered over posterior half of body. Eggs large, 0.045–0.054 by 0.027–0.045, few in number, and restricted to posterior body region. Excretory vesicle V-shaped.

Hosts: Sorex bendirii palmeri, S. palustrus navigator, and *S. obscurus permiliensis.*

Habitat: small intestine.

Locality: Cascade Range, Oregon, and Rocky Mountains, Montana.

Type specimen: U. S. Nat. Mus. Hel. Coll. No. 37383, paratype No. 37384.

Xiphidiotrema n. g.

Generic diagnosis: Troglotrematidae: small pyriform distomes with spinose cuticula. Oral sucker subterminal with a stylet imbedded in dorsal wall. Prepharynx absent, small pharynx and short esophagus present. Intestinal ceca extending into posterior half of body. Genital pore median, posterior to acetabulum. Cirrus sac small, on left. Cirrus and seminal vesicle present. Uterus confined posterior to ventral sucker, containing few eggs. Vitelline follicles large, restricted to posterior half of body. Eggs large. Excretory vesicle V-shaped.

Received for publication, January 19, 1953.

[1] Reed College Scholar, 1951–52; National Science Foundation Fellow, 1952–53.

Type species: Xiphidiotrema lockerae n. sp.

Although this fluke most certainly belongs in the group of trematodes generally assigned to the family TROGLOTREMATIDAE, it differs from them in the possession of a stylet in the adult stage, the anterior position of the testes, and the distribution of the vitellaria. Although it resembles *Nephrotrema,* Baer, in the position of the gonads, the present species is much smaller and occurs in the intestine rather than the kidney and its duct. Being more like *Sellacotyle,* Wallace, and *Nanophyetus,* Chapin, in these respects, *Xiphidiotrema* is placed in the subfamily NANOPHYETINAE which Wallace (1935) proposed for the small, intestinal trematodes of the family TROGLOTREMATIDAE. Life-history studies have demonstrated that members of that family have microcercous cercariae with a well developed stylet in the oral sucker. In the adult of *X. lockera,* an extremly small species, the presence of a stylet almost certainly is due to a retention of that structure during post-cercarial development.

SUMMARY

Xiphidiotrema lockerae, gen. et sp. nov., is described from shrews of the genus *Sorex.* It is characterized by small size, postacetabular genital pore, preacetabular testes, and a stylet in the adult stage. *Xiphidiotrema lockerae* is placed in the subfamily NANOPHYETINAE of the family TROGLOTREMATIDAE.

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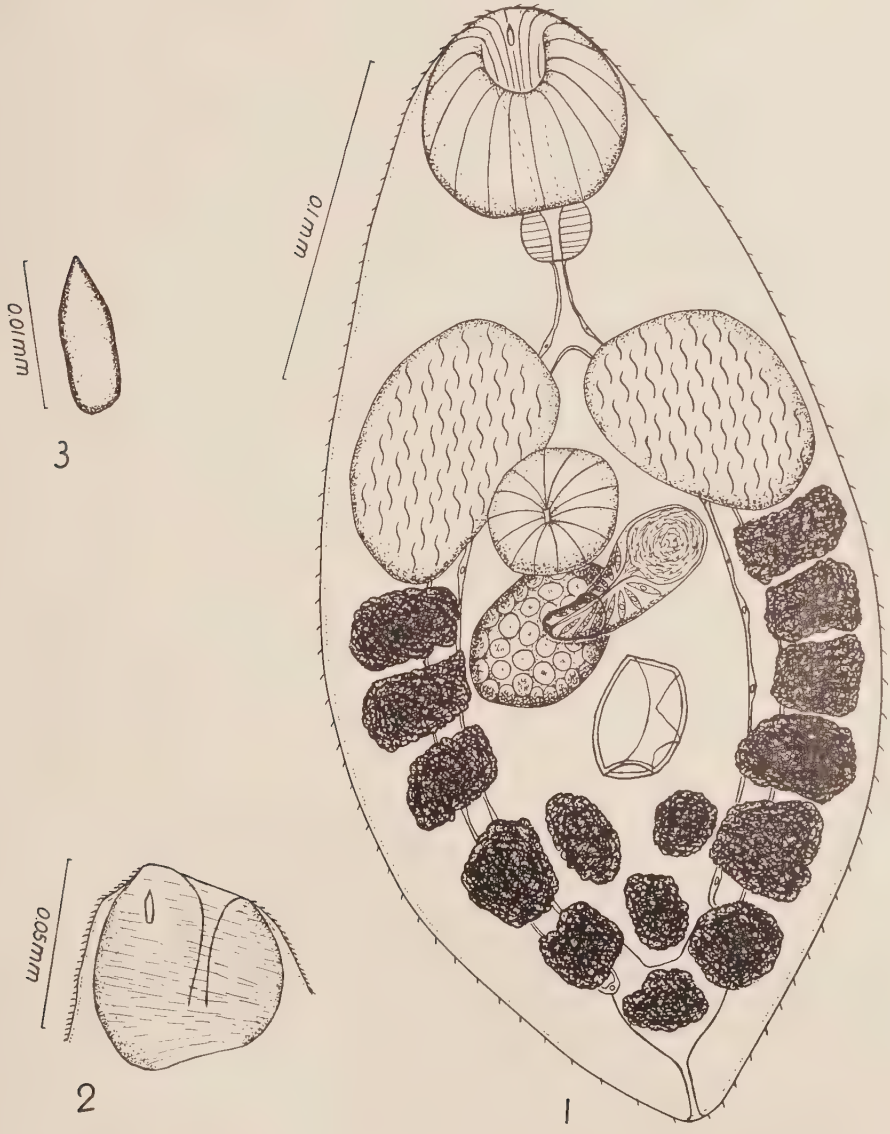
EXPLANATION OF PLATE

All drawings made with the aid of a camera lucida.

FIG. 1. *Xiphidiotrema lockerae,* n. g., n. sp.; ventral view of specimen containing only one egg. FIG. 2. Side view of oral sucker with stylet. FIG. 3. Stylet enlarged.

PLATE I



STUDIES ON THE HELMINTH FAUNA OF ALASKA. XV. SOME NOTES ON THE CYSTICERCUS OF *TAENIA POLYACANTHA* LEUCKART, 1856, FROM A VOLE (*MICROTUS OECONOMUS OPERARIUS* NELSON)

EVERETT L. SCHILLER*

While conducting parasitological studies on the Yukon delta during the latter part of July, 1952, the writer obtained a single female tundra vole, *Microtus oeconomus operarius* Nelson. Observations in this indicated that the microtine population was at an extremely low level of density. This vole was taken alive about 20 miles east of Igiak Bay, Alaska, and maintained in the laboratory for 63 days. At autopsy 47 cestode larvae of the genus *Taenia L.,* 1758, were found, unattached within the abdominal cavity. Forty-two of the larvae were allowed to remain in tap water (where they were quite active at room temperature) for six hours prior to preservation in alcohol-formalin-acetic acid solution. They were prepared as stained whole-mount specimens for morphological study. The scolices of all these larvae were invaginated when removed from the abdominal cavity of the vole and failed to evaginate either during relaxation in water or during fixation. The other five larvae were fed (with negative results, at 15 days) to a male domestic kitten weighing 1012 grams, the only carnivore available for experimental infection at the time.

Description of larva

Total length 8 to 12 mm.; maximum diameter 3 to 4 mm. Body divided into three regions: posterior tear-drop-shaped bladder, 6 mm. long by 3 mm. in diameter; dense parenchymatous middle region with well developed musculature, about 4 mm. long by 3 mm. in diameter; and muscular anterior region, containing invaginated scolex, about 2 mm. long by 1 mm. in diameter. Middle region overlaps apex of bladder. Margin of middle region roughly segmented. Scolex 0.7 mm. in diameter. Sucker spherical, about 0.250 mm. in diameter. Rostellum bears double row of from 44 to 48 hooks. Large hooks 0.210 mm. in length; small hooks 0.140 to 0.155 mm. in length.

DISCUSSION

Two species of *Taenia* (*T. tenuicollis* Rudophi, 1809, a common parasite of certain mustelids, and *T. crassiceps* Rudolphi, 1810, a parasite of the arctic fox, *Alopex lagopus innuitus* (Merriam)) whose larval stages are harbored by microtine rodents were reported from Alaska by Rausch (1952). The cysticerci of *T. tenuicollis* frequently occur in Alaskan voles and lemmings and are readily distinguished from the cestode larvae herein described on the basis of their size, location within the host (liver), and size and shape of rostellar hooks. The cysticerci of *T. crassiceps* are occasionally found in the thoracic and peritoneal cavities of microtine rodents in Europe, but are more frequently found in the subcutaneous tissues. Baer and Scheidegger (1946) discussed the subcutaneous tissue localization of the larval form of this species and the capacity of these larvae to multiply by budding. This characteristic of exogenous reproduction serves to distinguish the cysticerci of *T. crassiceps* from the *Taenia* larvae reported in this study; however, they are further

Received for publication, November 24, 1952.

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differentiated by much smaller size (2.5 to 3 mm. in length), a smaller scolex (0.520 to 0.540 mm.), and fewer rostellar hooks (30 to 32) which differ in size and shape (large hooks, 0.187 to 0.190 mm.; small hooks 0.133 to 0.136 mm.). Baer and Scheidegger (1946), in consideration of the hooks of *T. crassiceps,* stated that "La forme de ces crochets est absolument caractéristique de l'espéce." The hooks of a mature specimen of *T. crassiceps* from an arctic fox are shown in figure 3.

Another cestode parasite of European foxes, *T. polyacantha* Leuckart, 1856, whose larval stage has been found free in the body cavity of a microtine rodent, *Evotomys glareolus helveticus* Mill. (= *Clethrionomys glareolus helveticus* Schreber, 1780), has not been recorded from North America. The morphological features of the larvae obtained from *Microtus oeconomus operarius* in this study show close agreement with those of *T. polyacantha.* The rostellar hooks are identical in size and shape (Fig. 2). Although the numbers of hooks in *T. polyacantha* range from 52 to 60 compared to 44 to 48 in the larvae from the Alaskan vole, the latter numbers are apparently well within the possible limits of variation seen in other species of *Taenia.* It should be pointed out that the hook measurements for *T. polyacantha* as given by Wardle and McLeod (1952, p. 408) (larger hooks 0.058 mm.; smaller hooks 0.034 mm.) are apparently erroneous.

Baer (1932) stated that "La forme larvaire de *T. polyacantha* se présente tout á fait comme un plérocercoide . . . ces larves se trouvent libres dans la cavité du corps du Rongeur; il n'y a pas trace de membranes ou d'enveloppes larvaires ni de vésicule terminale." Although the living larvae, when removed from the rodent, had the gross appearance of plerocercoids similar to that figured by Baer (1932), subsequent relaxation in and absorption of water resulted in an inflation of the posterior vesicle. Determination of this vesicle when in a contracted state would be difficult if not impossible. This condition may account for these discrepant versions of the body forms. The writer considers his specimens to be *Taenia*-type cysticerci. While these cysticerci do not correspond exactly in morphological detail to the published descriptions of the European material, there appears to be sufficient agreement in body size, character of the scolex, size, shape and number of the rostellar hooks, and localization within the body of the host, to justify assignment of the Alaskan form to *T. polyacantha.* Three slides, containing specimens of *T. polyacantha* cysticerci have been deposited in the Helminthological Collection of the U. S. National Museum, No. 47896.

An example of an apparently anomalous larval development: An interesting example of joined larvae was observed among the 47 cysticerci obtained from this vole. The two individuals, otherwise identical with the other larvae, were found to be connected at their posterior ends by a small mass of dense tissue measuring 0.770 × 0.350 mm. (Fig. 4). Inasmuch as these larvae are connected by extra-larval tissue, this does not appear to be an example of exogenous budding as discussed and illustrated by Baer and Scheidegger (1946). Crusz (1948) described a process of annular constriction of the cysticercus of *T. pisiformis* Bloch, 1780, where two or more individuals are produced but the posterior individuals are without scolices. Since both of the larvae in this instance have undergone complete development, it is not possible to determine whether one was a result of asexual reproduction from the other or whether both originated and developed at the same time from the interconnecting tissue. This tissue is granular and nucleated and may have germinative

properties. A slide containing these specimens has been included among those deposited in the Helminthological Collection of the U. S. National Museum.

ACKNOWLEDGMENT

The writer wishes to take this opportunity to express his appreciation to Dr. Robert Rausch of this laboratory for identification of the specimen of *Microtus oeconomus operarius,* for supplying slides from his collection of cysticerci and adults of *T. crassiceps* and *T. tenuicollis,* and for the loan of the European specimens of adult *T. polyacantha* utilized as comparative material in this study.

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Explanation of Plate

Fig. 1. Cysticercus of *Taenia polyacantha* from an Alaskan vole, *Microtus oeconomus operarius.*

Fig. 2. Rostellar hooks of *T. polyacantha* cysticercus from *Microtus oeconomus operarius.*

Fig. 3. Rostellar hooks of *T. crassiceps* adult from an arctic fox, *Alopex lagopus innuitus.*

Fig. 4. An example of interconnected cysticerci of *T. polyacantha* from *Microtus oeconomus operarius.*

PLATE I

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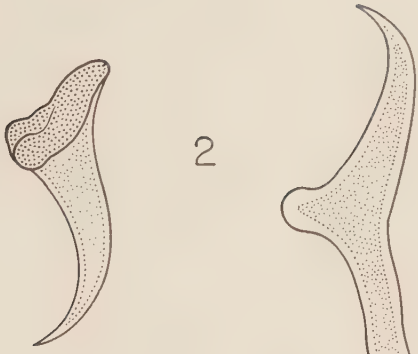

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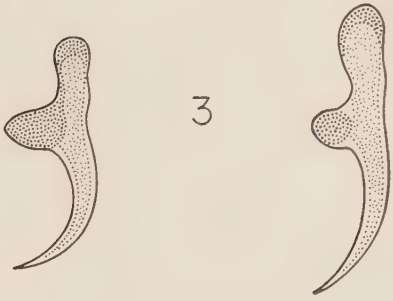

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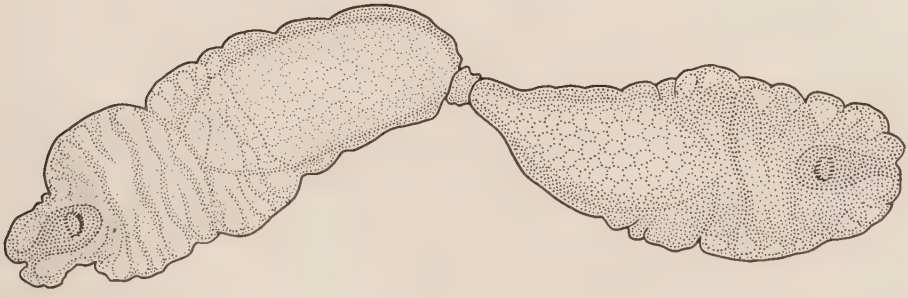

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A PRELIMINARY LIST OF COMMON NAMES FOR HELMINTHS

Many species of helminths are attracting attention because of their economic importance. Extension workers and others refuse to employ the Latin names which characterize scientific nomenclature. Unfortunately, names based on familiar English words are not well established. Consequently, confusion is prevalent in current usage.

In the hope of standardizing American usage, the Committee on Common Names of the ASP has selected from the literature those names most widely used. Since frequency of usage is the criterion, the style of the names suggested in this list is not uniform. We also feel that generic names may be employed as common names wherever applicable. This list is regarded as a tentative proposal and shall be subject to revision as soon as sufficient experience and the accumulation of suggestions warrant.

H. L. Van Volkenberg
D. B. McMullen
J. R. Christie
P. D. Harwood, *Chairman*

LIST OF COMMON NAMES OF HELMINTHS

PART I: NEMATODA

Acanthocheilonema perstans	Persistent filaria
Aelurostrongylus abstrusus	Cat lungworm
Ancylostoma braziliense	Creeping-eruption hookworm
Ancylostoma caninum	Dog hookworm
Ancylostoma duodenale	Old-world hookworm
Ancylostomidae	Hookworms
Anguina tritici	Wheat nematode
Aphelenchoides besseyi	Summer-dwarf nematode of strawberries
Aphelenchoides cocophilus	Coconut palm nematode
Aphelenchoides fragariae	Spring-dwarf nematode of strawberries
Aphelenchoides ritzema-bosi	Foliar nematode of chrysanthemums
Aphelenchoides olesistus	Foliar nematode of ferns and begonias
Ascaridae	Ascarids
	Large roundworms
Ascaridia	Large roundworms of birds
Ascaridia columbae	Large roundworm of pigeons
Ascaridia galli (syn. *A. lineata*)	Large roundworm of chickens
Ascaris equorum	Large roundworm of horses
Ascaris lumbricoides	Large roundworm of man
Ascaris lumbricoides var. *suis*	Large roundworm of swine
Ascaris vitulorum	Large roundworm of cattle
Ascarops strongylina and *Physocephalus sexalatus*	Thick stomach worms
Belonolaimus gracilis	Sting nematode
Bunostomum phlebotomum	Hookworm of cattle
Bunostomum trigonocephalum	Sheep hookworm
Capillaria spp.	Capillary worms
Capillaria annulata and *C. contorta*	Crop capillary worms
Capillaria columbae	Pigeon capillary worms
Chabertia ovina	Large-mouthed bowel worm
Cheilospirura hamulosa	Gizzard worm of fowls

Received for publication, November 16, 1952.

Choerostrongylus pudendotectus	(see *Metastrongylus elongatus* and *M. salmi*)
Cooperia spp.	Cooper's worms
Cooperia curticei	Cooper's worm of sheep
Cooperia pectinata, C. punctata and *C. oncophora*	Cooper's worms of cattle
Crassisoma urosubulatum	Hookworm of swine
Criconema spp. and *Criconemoides* spp.	Ring nematodes
Dictyocaulus arnfieldi	Horse lungworm
Dictyocaulus filaria	Thread lungworm of sheep
Dictyocaulus viviparus	Lungworm of cattle
Dioctophyme renale	Giant kidney worm
Dracunculus medinensis	Guinea worm
Dirofilaria immitis	Dog heart worm
Dispharynx spiralis	Spiral stomach worm
Ditylenchus angustus	Rice nematode
Ditylenchus destructor	Potato-rot nematode
Ditylenchus dipsaci	Bulb and stem nematode
Dolichodorus heterocephalus	Awl nematode
Enterobius vermicularis	Human pinworm
Filariidae	Filaria
Gongylonema pulchrum	Gullet worm
Gongylonema ingluvicola	Gullet worm of fowls
Gordiacea	Horse-hair worm
Habronema muscae, H. megastoma, and *H. microstoma*	Stomach worms of horses
Haemonchus contortus	Twisted stomach worm
Helicotylenchus spp. and *Rotylenchus* spp.	Spiral nematodes
Heterakis gallinae	Common cecal worm
Heterodera rostochiensis	Golden nematode of potatoes
Heterodera schachtii	Sugar-beet nematode
Hoplolaimus spp.	Lance nematodes
Hyostrongylus rubidus	Red stomach worm of swine
Loa loa	Loa
Mansonella ozzardi	Ozzard's filaria
Meloidogyne spp.	Root-knot nematodes
Metastrongylidae	Lungworms
Metastrongylus elongatus, M. salmi and *Choerostrongylus pudendotectus*	Swine lungworms
Muellerius capillaris	Hair lungworm
Necator americanus	American hookworm
Nemathelminthes	Roundworms
Nematoda	Nematodes
Nematodirus filicollis and *N. spathiger*	Thread-necked strongyles
Oesophagostomum spp.	Nodular worms
Oesophagostomum apiostomum	Monkey nodular worm
Oesophagostomum columbianum	Sheep nodular worm
Oesophagostomum dentatum, O. longicaudum, O. brevicaudum and *O. georgianum*	Nodular worms of swine
Oesophagostomum radiatum	Nodular worm of cattle
Oesophagostomum venulosum	Straight nodular worm
Onchocerca cervicalis	Neck threadworm
Onchocerca volvulus	Blinding filaria
Ornithostrongylus quadriradiatus	Intestinal strongyle of pigeons
Oslerus osleri	Dog lungworm
Ostertagia circumcincta and *O. trifurcata*	Brown stomach worms of sheep
Ostertagia ostertagi	Brown stomach worm of cattle
Oxyspirura mansoni	Eyeworm of poultry
Oxyuris equi	Horse pinworm
Physocephalus sexalatus and *Ascarops strongylina*	See *Ascarops strongylina*
Pratylenchus spp.	Pin nematodes
Pratylenchus spp.	Meadow nematodes
Pratylenchus leiocephalus	Smooth-headed meadow nematode

Pratylenchus scribneri	Scribner's meadow nematode
Probstmayria vivipara	Minute pinworm
Radopholus similis	Burrowing nematode
Rotylenchus spp. and *Helicotylenchus* spp.	Spiral nematodes
Spirocerca lupi	Esophageal worm of dogs
Stephanurus dentatus	Kidney worm of swine
Strongyloides spp.	Intestinal threadworms
Strongyloides ransomi	Intestinal threadworms of swine
Strongyloides stercoralis	Human intestinal threadworms
Strongylus spp.	Large strongyles
Strongylus edentatus	Large toothless strongyle
Strongylus equinus	Double-toothed strongyle
Strongylus vulgaris	Single-toothed strongyle
Syngamidae	Forked worms
Syngamus laryngeus	Throat worm
Syngamus trachea	Gapeworm of fowls
Tetrameres americana	Globular stomach worm
Thelazia callipaeda	Oriental eye-worm
Thelazia californiensis	California eye-worm
Toxocara canis, T. cati and *Toxascaris leonina*	Large roundworms of carnivores Ascarids of carnivores
Trichinella spiralis	Trichina worm
Trichodorus spp.	Stubby-root nematodes
Trichonema spp. and allies	Small strongyles
Trichostrongylus spp.	Hairworms
Trichostrongylus axei	Minute stomach worm
Trichuris spp.	Whipworms
Trichuris ovis	Ruminant whipworm
Trichuris trichiura	Human whipworm
Trichuris vulpis	Dog whipworm
Turbatrix aceti v. *aceti*	Vinegar eelworm
Tylenchulus semi-penetrans	Citrus nematode
Wuchereria bancrofti	Bancroft's filaria
Wuchereria malayi	Malayan filaria
Xiphinema spp.	Dagger nematodes

PART II: CESTODA

Anoplocephala magna	Large horse tapeworm
Anoplocephala perfoliata	Perfoliate tapeworm
Cestoda	Tapeworms Cestodes
Coenurus cerebralis	Gid bladder worm
Cysticercus bovis	Beef bladder worm
Cysticercus cellulosae	Pork bladder worm
Cysticercus tenuicollis	Thin-necked bladder worm
Davainea proglottina	Minute tapeworm
Diphyllobothrium latum	Fish tapeworm of man
Diphyllobothrium cordatum	Heart-headed tapeworm
Diphyllobothrium mansoni	Manson's tapeworm
Diplogonoporus grandis	Double-pored giant tapeworm
Dipylidium caninum	Double-pored dog tapeworm
Drepanidotaenia lanceolata	Lanceolate tapeworm
Echinococcus granulosis	Hydatid tapeworm
Helictometra giardi	Single-pored ruminant tapeworm
Hymenolepis cantaniana	Branching tapeworm
Hymenolepis carioca	Thread tapeworm
Hymenolepis diminuta	Rat tapeworm
Hymenolepis nana	Dwarf tapeworm
Moniezia expansa and *M. benedeni*	Double-pored ruminant tapeworm
Multiceps multiceps	Gid tapeworm
Paranoplocephala mamillana	Dwarf tapeworm of horses
Raillietina celebenis	Celebes tapeworm

Raillietina cesticillus	Broad-headed tapeworm
Raillietina echinobothrida	Nodular tapeworm
Raillietina madagascariensis	Madagascar tapeworm
Raillietina tetragona	Oval-suckered tapeworm
Taenia africana	African tapeworm
Taenia saginata	Beef tapeworm
Taenia solium	Pork tapeworm
Thysanosoma actinioides	Fringed tapeworm

PART III: TREMATODA

Amphistomata	Amphistomes
Aporocotylidae	Fish blood flukes
Brachylaemidae	Harmostomes
Bucephalidae	Gasterostomes
Clonorchis sinensis	Chinese liver flukes
Collyriclum faba	Cystic fluke
Dicrocoelium dendriticum	Lancet fluke
Echinostomidae	Echinostomes
Fasciola gigantica	Giant liver fluke
Fasciola hepatica	Common liver fluke
Fascioloides magna	Large liver fluke
Fasciolopsis buski	Large intestinal fluke
Haematoloechus spp.	Frog lung flukes
Monostomata	Monostomes
Paramphistomidae	Amphistomes
Paragonimus westermanii	Human lung fluke
Polystomidae	Polystomes
Psilostomidae	Psilostomes
Schistosoma bovis	Bovine blood fluke
Schistosoma haematobium	Vesical blood fluke
Schistosoma japonicum	Oriental blood fluke
Schistosoma mansoni	Manson's blood fluke
Schistosomatidae	Schistosomes Blood flukes
Spirorchidae	Turtle blood flukes
Strigeoidea	Strigeids
Trematoda	Trematodes Flukes

PART IV: ACANTHOCEPHALA

Acanthocephala	Thorny-headed worms
Macracanthorynchus hirudinaceus	Thorny-headed worm of swine
Moniliformis dubius	Thorny-headed worm of rats

A NEW DIGENETIC TREMATODE (*CEPHALOUTERINA* DICAMPTODONI N. G., N. SP., PLEUROGENETINAE) FROM THE PACIFIC GIANT SALAMANDER

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During the summers of 1950 and 1951, the first author examined about 50 specimens of the Pacific giant salamander, *Dicamptodon ensatus* (Eschscholtz), collected near Portland, Oregon. About half of these were found to harbor a species of intestinal fluke with peculiar anatomical features. Although resembling trematodes assigned to the subfamilies PLEUROGENETINAE and CEPHALOGONIMINAE in some respects, this species could not be placed in any of their genera. The authors therefore propose for it the new genus *Cephalouterina* with *Cephalouterina dicamptodoni* n. sp. as type species.

The specimens were fixed in either Bouin's fluid, formalin-alcohol-acetic or corrosive-acetic acid solution. They were stained with borax carmine or Ehrlich's hematoxylin, cleared in xylene or terpineol, and mounted in Clarite. Serial sections were stained with Galigher's alum hematoxylin and eosin. All measurements are in millimeters with averages in parentheses.

We wish to express our thanks to Mr. Kenneth Neiland for his help in obtaining the salamanders utilized in this study and to Dr. R. M. Cable for critical reading of this paper.

Cephalouterina dicamptodoni, n. g., n. sp. (Fig. 1–3)

Specific diagnosis: Body ellipsoidal, 0.96–1.60 (1.34) long by 0.45–0.75 (0.57) wide. Cuticula spinose overall. Oral sucker subterminal, 0.16–0.20 (0.18) long and 0.18–0.22 (0.20) wide; prepharynx short. Pharynx 0.06–0.09 in diameter, esophagus about 0.18 long. Intestinal ceca short and expanded, ending at about mid-acetabular level. Ventral sucker 0.18–0.28 (0.22) in diameter, slightly anterior to mid-line of body. Testes opposite, just within posterior half of body and measuring 0.20–0.35 (0.28) long and 0.11–0.18 (0.15) wide. Cirrus sac on left of mid-line, 0.30–0.45 long by 0.04–0.07 wide, and extending anteriorly from near the intestinal bifurcation to the level of the oral sucker Seminal vesicle bipartite, with small anterior portion somewhat resembling a pars prostatica, remainder sinuous and 0.10–0.18 in length and 0.04–0.06 wide. A few prostate cells surround the cirrus. Genital pore dorsal and lateral, to left side of oral sucker. Genital atrium a shallow depression. Ovary anterior and median to right testis, reaching to about equator of acetabulum; varying in shape but usually ellipsoidal, measuring 0.12–0.25 (0.17) long and 0.09–0.14 (0.11) wide. Seminal receptacle between testes, 0.08–0.13 long by 0.02–0.06 wide, and constricted with Laurer's canal leaving smaller portion as a sinuous tube opening dorsally. Vitellaria somewhat variable in extent generally with a compact mass of follicles on each side anterior to intestinal ceca and more scattered lateral ones posterior to ceca and extending to a point alongside or even posterior to testes. Follicles usually meet dorsally anterior to ventral sucker. From oötype region between the testes, the uterus passes anteriorly to left side of acetabulum and has a few loose coils anterior to each intestinal cecum. Eggs not numerous, measuring 0.038–0.050 (0.041) by 0.017–0.022 (0.020); thin shelled, light brown, with prominent operculum and short polar spine. Excretory vesicle

Received for publication, January 21, 1953.
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Y-shaped, with long, unbranched stem bifurcating between testes to form short arms which taper to narrow main excretory tubules passing anteriorly and laterally around intestinal ceca.

Host: Dicamptodon ensatus (Eschscholtz).

Habitat: Small intestine.

Locality: Cascade Range, Oregon, U. S. A.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 47554.

Cephalouterina, n. g.

Generic diagnosis: Pleurogenetinae: Small ellipsoidal distomes, cuticula spinose. Oral sucker subterminal; short prepharynx, pharynx, and esophagus present. Intestinal ceca reduced. Cirrus sac large and elongate. Genital pore dorsal at the level of the oral sucker. Uterus not extending posterior to gonads, confined mainly to region anterior to intestinal ceca. Testes opposite, well removed from posterior end of body. Ovary anterior to testes. Seminal receptacle and Laurer's canal present. Vitellarian follicles both anterior and posterior to acetabulum. Eggs medium-sized with short polar spine. Excretory vesicle Y-shaped with short arms.

Type species: Cephalouterina dicamptodoni, n. sp.

DISCUSSION

While in certain respects this fluke resembles the genera *Cephalogonimus,* Poirier, *Emoleptalea,* Looss, *Cephalotrema,* Baer, and *Prosthogonimus,* Lühe, it is probably most closely related to *Sonsinotrema,* Balozet and Callot, because of similar placement of gonads, length of intestinal ceca, shape and location of the cirrus sac, shape of the excretory vesicle, and the almost identical configuration of the oötype region. It is different from the other genera listed in one or more of these respects. *Cephalouterina* is distinguished from *Sonsinotrema* by its unbranched stem of the excretory vesicle, the distribution of vitellaria, the position of the genital pore, and the extent of the uterus.

The disposition of the genus *Cephalouterina* in a subfamily or family is difficult at present because the excretory pattern and life history are unknown and comparisons in these respects cannot be made. If one considers the length of the intestinal ceca, the placement of the gonads, and the configuration of the oötype region, it would appear that this genus belongs in the subfamily PLEUROGENETINAE of the family LECITHODENDRIIDAE, although the dorsal position of the genital pore is more characteristic of the CEPHALOGONIMINAE of the family PLAGIORCHIIDAE. However, the position of the sex opening is highly variable in the digenetic trematodes and the genus is otherwise more like *Sonsinotrema* and related forms. It therefore seems advisable to place *Cephalouterina* in the PLEUROGENETINAE pending further information concerning the excretory pattern and life history.

SUMMARY

Cephalouterina dicamptodoni n. g., n. sp. is described from the Pacific giant salamander, *Dicamptodon ensatus.* The genus is characterized by a dorsal genital pore near the anterior end, short intestinal ceca, and a short uterus not extending posterior to the gonads. *Cephalouterina dicamptodoni* is placed in the subfamily PLEUROGENETINAE, family LECITHODENDRIIDAE.

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EXPLANATION OF PLATE

FIG. 1. *Cephalouterina dicamptodoni*, n. g., n. sp., holotype, ventral view.
FIG. 2. Oötype region, paratype, dorsal view.
FIG. 3. Egg.

PLATE I